

I claim:

1. A method of conferring disease resistance to a transgenic plant, the method comprising

5 a) providing a transgenic plant comprising a recombinant DNA molecule comprising a promoter operably linked to a DNA sequence comprising, in the 5' to 3' direction,

i) a sequence complementary to a coding sequence for a heterologous polypeptide capable of conferring disease resistance;

10 ii) a sequence complementary to an internal ribosome entry site;

iii) a 3' UTR of a first positive strand single-stranded RNA virus; and

b) growing the transgenic plant,

15 whereby resistance is conferred to infection from a second positive strand single-stranded RNA virus.

2. The method of conferring disease resistance to a transgenic plant of claim 1, wherein the promoter is selected from the group consisting of a constitutive promoter and an inducible promoter.

20 3. The method of conferring disease resistance to a transgenic plant of claim 2, wherein the promoter is a constitutive promoter.

4. The method of conferring disease resistance to a transgenic plant of claim 3, wherein the constitutive promoter is a eukaryotic constitutive promoter selected from the group consisting of a cauliflower mosaic virus 35S promoter, a blueberry red ringspot virus promoter, a ubiquitin gene promoter, an actin gene promoter, an NeIF-4A10 promoter, a maize Adh1-based pEmu promoter, a barley leaf thionin BTH6 promoter, a cassava vein mosaic virus promoter, a sugarcane bacilliform badnavirus promoter and a histone gene promoter.

25 5. The method of conferring disease resistance to a transgenic plant of claim 4, wherein the eukaryotic constitutive promoter is a cauliflower mosaic virus 35S promoter.

6. The method of conferring disease resistance to a transgenic plant of claim 5, wherein the cauliflower mosaic virus 35S promoter comprises the sequence:
AGATTAGCCTTTTCAATTTTCAGAAAGAATGCTAACCCACAGATGGTTAGA
GAGGCTTACGCAGCAGGTCTCATCAAGACGATCTACCCGAGCAATAATCT

CCAGGAAATCAAATACCTTCCCAAGAAGGTAAAGATGCAGTCAAAAGAT
 TCAGGACTAACTGCATCAAGAACACAGAGAAAGATATATTTCTCAAGATC
 AGAAGTACTATTCCAGTATGGACGATTCAAGGCTTGCTTCACAAACCAAG
 GCAAGTAATAGAGATTGGAGTCTCTAAAAAGGTAGTTCCCACTGAATCAA
 5 AGGCCATGGAGTCAAAGATTCAAATAGAGGACCTAACAGAACTCGCCGTA
 AAGACTGGCGAACAGTTCATACAGAGTCTCTTACGACTCAATGACAAGAA
 GAAAATCTTCGTCAACATGGTGGAGCACGACACACTTGTCTACTCCAAAA
 ATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTGAGACTTTTCAA
 CAAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTGCCAGCTATCTGT
 10 CACTTTATTGTGAAGATAGTGGAAAAGGAAGGTGGCTCCTACAAATGCCA
 TCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTG
 GTCCCAAAGATGGACCCCCACCCACGAGGAGCATCGTGGAAAAAGAAGA
 CGTTCCAACCACGTCTTCAAAGCAAGTGGATTGATGTGATATCTCCACTGA
 CGTAAGGGATGACGCACAATCCCACTATCCTTCGCAAGACCCTTCCTCTAT
 15 ATAAGGAAGTTCATTTCAATTTGGAGAGAACACG (SEQ ID NO: 3).

7. The method of conferring disease resistance in a transgenic cell of claim 1, wherein the coding sequence for a heterologous polypeptide encodes a polypeptide selected from the group consisting of a cell toxin and a viral polypeptide.

8. The method of conferring disease resistance in a transgenic cell of claim 7, wherein the viral polypeptide is a viral coat protein polypeptide.

9. The method of conferring disease resistance to a transgenic plant of claim 1, wherein the sequence complementary to an IRES is a sequence complementary to an IRES selected from the group consisting of a picornavirus IRES, a foot-and-mouth disease virus IRES, an encephalomyocarditis virus IRES, a hepatitis
 25 A virus IRES, a hepatitis C virus IRES, a human rhinovirus IRES, a poliovirus IRES, a swine vesicular disease virus IRES, a turnip mosaic potyvirus IRES, a human fibroblast growth factor 2 mRNA IRES, a pestivirus IRES, a Leishmania RNA virus IRES, a Moloney murine leukemia virus IRES a human rhinovirus 14 IRES, an aphthovirus IRES, a human immunoglobulin heavy chain binding protein mRNA
 30 IRES, a *Drosophila* Antennapedia mRNA IRES, a human fibroblast growth factor 2 mRNA IRES, a hepatitis G virus IRES, a tobamovirus IRES, a vascular endothelial growth factor mRNA IRES, a Coxsackie B group virus IRES, a c-myc protooncogene mRNA IRES, a human MYT2 mRNA IRES, a human parechovirus type 1 virus IRES, a human parechovirus type 2 virus IRES, a eukaryotic initiation factor 4GI

mRNA IRES, a Plautia stali intestine virus IRES, a Theiler's murine encephalomyelitis virus IRES, a bovine enterovirus IRES, a connexin 43 mRNA IRES, a homeodomain protein Gtx mRNA IRES, an AML1 transcription factor mRNA IRES, an NF-kappa B repressing factor mRNA IRES, an X-linked inhibitor of apoptosis mRNA IRES, a cricket paralysis virus RNA IRES, a p58(PITSLRE) protein kinase mRNA IRES, an ornithine decarboxylase mRNA IRES, a connexin-32 mRNA IRES, a bovine viral diarrhea virus IRES, an insulin-like growth factor I receptor mRNA IRES, a human immunodeficiency virus type 1 gag gene IRES, a classical swine fever virus IRES, a Kaposi's sarcoma-associated herpes virus IRES, a short IRES selected from a library of random oligonucleotides, a Jembrana disease virus IRES, an apoptotic protease-activating factor 1 mRNA IRES, a Rhopalosiphum padi virus IRES, a cationic amino acid transporter mRNA IRES, a human insulin-like growth factor II leader 2 mRNA IRES, a giardiavirus IRES, a Smad5 mRNA IRES, a porcine teschovirus-1 talfan IRES, a *Drosophila* Hairless mRNA IRES, an hSNM1 mRNA IRES, a Cbfa1/Runx2 mRNA IRES, an Epstein-Barr virus IRES, a hibiscus chlorotic ringspot virus IRES, a rat pituitary vasopressin V1b receptor mRNA IRES, and a human hsp70 mRNA IRES.

10. The method of conferring disease resistance to a transgenic plant of claim 9, wherein the sequence complementary to an internal ribosome entry site is a sequence complementary to a picornavirus internal ribosome entry site.

11. The method of conferring disease resistance to a transgenic plant of claim 10, wherein the sequence complementary to a picornavirus internal ribosome entry site comprises the sequence:

TTATCATCGTGTTTTTCAAAGGAAAACACGTCCCCGTGGTTCGGGGGGCC
 TAGACGTTTTTTTAACCTCGACTAAACACATGTAAAGCATGTGCACCGAG
 GCCCCAGATCAGATCCCATAACAATGGGGTACCTTCTGGGCATCCTTCAGCC
 CCTTGTTGAATACGCTTGAGGAGAGCCATTTGACTCTTTCCACAACCTATCC
 AACTCACAACGTGGCACTGGGGTTGTGCCGCCTTTGCAGGTGTATCTTATA
 CACGTGGCTTTTGGCCGCAGAGGCACCTGTCGCCAGGTGGGGGGTTCCGC
 TGCCTGCAAAGGGTCGCTACAGACGTTGTTTGTCTTCAAGAAGCTTCCAGA
 GGAAGTCTTCCTTCACGACATTCAACAGACCTTGCATTCCTTTGGCGAGA
 GGGGAAAGACCCCTAGGAATGCTCGTCAAGAAGACAGGGCCAGGTTTCC
 GGGCCCTCACATTGCCAAAAGACGGCAATATGGTGGAAAATCACATATAG

ACAAACGCACACCGGCCTTATTCCAAGCGGCTTCGGCCAGTAACGTTAGG
GGGGGGGGAGGGAGAGGGGCGGAATT (SEQ ID NO: 6).

12. The method of conferring disease resistance to a transgenic plant of claim 1, wherein the 3' UTR of a first positive strand single-stranded RNA virus is a 3' UTR of a first positive strand single-stranded RNA virus having no DNA stage.

13. The method of conferring disease resistance to a transgenic plant of claim 12, wherein the 3' UTR of a first positive strand single-stranded RNA virus having no DNA stage is a 3' UTR of a first bromovirus.

14. The method of conferring disease resistance to a transgenic plant of claim 13, wherein the 3' UTR of a first bromovirus is a 3' UTR of a first Cowpea chlorotic mottle virus.

15. The method of conferring disease resistance to a transgenic plant of claim 14, wherein a DNA copy of the 3' UTR of a first Cowpea chlorotic mottle virus comprises the sequence:

- AGTGCCCGCTGAAGAGCGTTACACTAGTGTGGCCTACTTGAAGGCTAGTT
ATAACCGTTTCTTTAAACGGTAATCGTTGTTGAAACGTCTTCCTTTTACAA
GAGGATTGAGCTGCCCTTGGGTTTTACTCCTTGAACCCTTCGGAAGAAGCTC
TTTGGAGTTCGTACCAGTACCTCACATAGTGAGGTAATAAGACTGGTGGG
CAGCGCCTAGTCGAAAGACTAGGTGATCTCTAAGGAGACC (SEQ ID NO:
8).

16. The method of conferring disease resistance to a transgenic plant of claim 1, further comprising a sequence complementary to an intron.

17. The method of conferring disease resistance to a transgenic plant of claim 1, further comprising a transcription termination signal.

18. The method of conferring disease resistance to a transgenic plant of claim 1, wherein the plant is a dicotyledonous plant.

19. The method of conferring disease resistance to a transgenic plant of claim 19, wherein the dicotyledonous plant is a *Nicotiana* plant.

20. The method of conferring disease resistance to a transgenic plant of claim 20, wherein the *Nicotiana* plant is a *Nicotiana benthamiana* plant.

21. The method of conferring disease resistance to a transgenic plant of claim 1, wherein the second positive strand single-stranded RNA virus is a positive strand single-stranded RNA virus having no DNA stage.

22. The method of conferring disease resistance to a transgenic plant of claim 21, wherein the second positive strand single-stranded RNA virus having no DNA stage is selected from the group consisting of a positive strand single-stranded RNA plant virus having no DNA stage and a positive single-stranded RNA animal virus having no DNA stage.

23. The method of conferring disease resistance to a transgenic plant of claim 22, wherein the second positive strand single-stranded RNA plant virus having no DNA stage is selected from the group consisting of a second Bromovirus, a Tobacco etch virus, a Tobacco vein mottle virus, and a Pepper mottle virus.

24. The method of conferring disease resistance to a transgenic plant of claim 23, wherein the second Bromovirus is selected from a second Cowpea chlorotic mottle virus and a second Brome mosaic virus.

25. The method of conferring disease resistance to a transgenic plant of claim 23, wherein the second Bromovirus is a second Cowpea chlorotic mottle virus.

26. The method of conferring disease resistance to a transgenic plant of claim 1, wherein the molar concentration ratio of heterologous polypeptide in a cell provided a stimulus relative to a cell not provided a stimulus is at least about 50:1.

27. The method of conferring disease resistance to a transgenic plant of claim 26, wherein the molar concentration ratio of heterologous polypeptide in a cell provided a stimulus relative to a cell not provided a stimulus is at least about 100:1.

28. The method of conferring disease resistance to a transgenic plant of claim 27, wherein the molar concentration ratio of heterologous polypeptide in a cell provided a stimulus relative to a cell not provided a stimulus is at least about 1000:1.

29. The method of conferring disease resistance to a transgenic plant of claim 28, wherein the molar concentration ratio of heterologous polypeptide in a cell provided a stimulus relative to a cell not provided a stimulus is at least about 10,000:1.

30. A method of producing a heterologous polypeptide in a transgenic plant, the method comprising:

- a) providing a transgenic plant comprising a recombinant DNA molecule comprising a promoter operably linked to a DNA sequence comprising, in the 5' to 3' direction,
 - i) a sequence complementary to a coding sequence for a heterologous polypeptide;

- ii) a sequence complementary to an internal ribosome entry site;
- iii) a 3' UTR of a first positive strand single-stranded RNA virus;
- 5 b) growing the transgenic plant; and
- c) providing a stimulus to the transgenic plant for synthesis of an RNA complementary to an RNA transcript of the recombinant DNA.

31. The method of producing a heterologous polypeptide in a transgenic plant of claim 30, wherein the promoter is selected from the group consisting of a constitutive promoter and an inducible promoter.

32. The method of producing a heterologous polypeptide in a transgenic plant of claim 31, wherein the promoter is a constitutive promoter.

33. The method of producing a heterologous polypeptide in a transgenic plant of claim 32, wherein the constitutive promoter is a eukaryotic constitutive promoter selected from the group consisting of a cauliflower mosaic virus 35S promoter, a blueberry red ringspot virus promoter, a ubiquitin gene promoter, an actin gene promoter, an NeIF-4A10 promoter, a maize Adh1-based pEmu promoter, a barley leaf thionin BTH6 promoter, a cassava vein mosaic virus promoter, a sugarcane bacilliform badnavirus promoter and a histone gene promoter.

34. The method of producing a heterologous polypeptide in a transgenic plant of claim 33, wherein the eukaryotic constitutive promoter is a cauliflower mosaic virus 35S promoter.

35. The recombinant DNA molecule of claim 34, wherein the cauliflower mosaic virus 35S promoter comprises the sequence:

25 AGATTAGCCTTTTCAATTTTCAGAAAGAATGCTAACCCACAGATGGTTAGA
GAGGCTTACGCAGCAGGTCTCATCAAGACGATCTACCCGAGCAATAATCT
CCAGGAAATCAAATACCTTCCCAAGAAGGTTAAAGATGCAGTCAAAAGAT
TCAGGACTAACTGCATCAAGAACACAGAGAAAGATATATTTCTCAAGATC
AGAAGTACTATTCCAGTATGGACGATTCAAGGCTTGCTTCACAAACCAAG
30 GCAAGTAATAGAGATTGGAGTCTCTAAAAAGGTAGTTCCCACTGAATCAA
AGGCCATGGAGTCAAAGATTCAAATAGAGGACCTAACAGAACTCGCCGTA
AAGACTGGCGAACAGTTCATACAGAGTCTCTTACGACTCAATGACAAGAA
GAAAATCTTCGTCAACATGGTGGAGCACGACACACTTGTCTACTCCAAAA
ATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTGAGACTTTTCAA

CAAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTGCCAGCTATCTGT
 CACTTTATTGTGAAGATAGTGGAAAAGGAAGGTGGCTCCTACAAATGCCA
 TCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTG
 GTCCCAAAGATGGACCCCCACCCACGAGGAGCATCGTGGAAAAAGAAGA
 5 CGTTCCAACCACGTCTTCAAAGCAAGTGGATTGATGTGATATCTCCACTGA
 CGTAAGGGATGACGCACAATCCCACTATCCTTCGCAAGACCCTTCCTCTAT
 ATAAGGAAGTTCATTTCAATTTGGAGAGAACACG (SEQ ID NO: 3).

36. The method of producing a heterologous polypeptide in a transgenic cell of claim 30, wherein the coding sequence for a heterologous polypeptide encodes
 10 a polypeptide selected from the group consisting of a hormone, an enzyme, a cell toxin, a viral polypeptide, a cell surface polypeptide, and an intracellular polypeptide.

37. The method of producing a heterologous polypeptide in a transgenic plant of claim 30, wherein the sequence complementary to an IRES is a sequence complementary to an IRES selected from the group consisting of a picornavirus IRES,
 15 a foot-and-mouth disease virus IRES, an encephalomyocarditis virus IRES, a hepatitis A virus IRES, a hepatitis C virus IRES, a human rhinovirus IRES, a poliovirus IRES, a swine vesicular disease virus IRES, a turnip mosaic potyvirus IRES, a human fibroblast growth factor 2 mRNA IRES, a pestivirus IRES, a Leishmania RNA virus IRES, a Moloney murine leukemia virus IRES a human rhinovirus 14 IRES, an
 20 aphthovirus IRES, a human immunoglobulin heavy chain binding protein mRNA IRES, a *Drosophila* Antennapedia mRNA IRES, a human fibroblast growth factor 2 mRNA IRES, a hepatitis G virus IRES, a tobamovirus IRES, a vascular endothelial growth factor mRNA IRES, a Coxsackie B group virus IRES, a c-myc protooncogene mRNA IRES, a human MYT2 mRNA IRES, a human parechovirus type 1 virus
 25 IRES, a human parechovirus type 2 virus IRES, a eukaryotic initiation factor 4GI mRNA IRES, a *Plautia stali* intestine virus IRES, a Theiler's murine encephalomyelitis virus IRES, a bovine enterovirus IRES, a connexin 43 mRNA IRES, a homeodomain protein Gtx mRNA IRES, an AML1 transcription factor mRNA IRES, an NF-kappa B repressing factor mRNA IRES, an X-linked inhibitor of
 30 apoptosis mRNA IRES, a cricket paralysis virus RNA IRES, a p58(PITSLRE) protein kinase mRNA IRES, an ornithine decarboxylase mRNA IRES, a connexin-32 mRNA IRES, a bovine viral diarrhea virus IRES, an insulin-like growth factor I receptor mRNA IRES, a human immunodeficiency virus type 1 gag gene IRES, a classical swine fever virus IRES, a Kaposi's sarcoma-associated herpes virus IRES, a short

IRES selected from a library of random oligonucleotides, a Jembrana disease virus IRES, an apoptotic protease-activating factor 1 mRNA IRES, a Rhopalosiphum padi virus IRES, a cationic amino acid transporter mRNA IRES, a human insulin-like growth factor II leader 2 mRNA IRES, a giardiavirus IRES, a Smad5 mRNA IRES, a
 5 porcine teschovirus-1 talfan IRES, a *Drosophila* Hairless mRNA IRES, an hSNM1 mRNA IRES, a Cbfa1/Runx2 mRNA IRES, an Epstein-Barr virus IRES, a hibiscus chlorotic ringspot virus IRES, a rat pituitary vasopressin V1b receptor mRNA IRES, and a human hsp70 mRNA IRES.

38. The method of producing a heterologous polypeptide in a transgenic
 10 plant of claim 37, wherein the sequence complementary to an internal ribosome entry site is a sequence complementary to a picornavirus internal ribosome entry site.

39. The method of producing a heterologous polypeptide in a transgenic plant of claim 38, wherein the sequence complementary to a picornavirus internal ribosome entry site comprises the sequence:

15 TTATCATCGTGTTTTTCAAAGGAAAACACGTCCCCGTGGTTCGGGGGGGCC
 TAGACGTTTTTTTAACTCGACTAAACACATGTAAAGCATGTGCACCGAG
 GCCCCAGATCAGATCCCATAACAATGGGGTACCTTCTGGGCATCCTTCAGCC
 CCTTGTTGAATACGCTTGAGGAGAGCCATTTGACTCTTTCCACAACATATCC
 AACTCACAAACGTGGCACTGGGGTTGTGCCGCCTTTGCAGGTGTATCTTATA
 20 CACGTGGCTTTTGGCCGCAGAGGCACCTGTCCGAGGTGGGGGGTTCCGC
 TGCCTGCAAAGGGTCGCTACAGACGTTGTTTGTCTTCAAGAAGCTTCCAGA
 GGAAGTCTTCCTTCACGACATTCAACAGACCTTGCATTCTTTGGCGAGA
 GGGGAAAGACCCCTAGGAATGCTCGTCAAGAAGACAGGGCCAGGTTTCC
 GGGCCCTCACATTGCCAAAAGACGGCAATATGGTGGAAAATCACATATAG
 25 ACAAACGCACACCGGCCTTATTCCAAGCGGCTTCGGCCAGTAACGTTAGG
 GGGGGGGGAGGGAGAGGGGCGGAATT (SEQ ID NO: 6).

40. The method of producing a heterologous polypeptide in a transgenic
 plant of claim 30, wherein the 3' UTR of a first positive strand single-stranded RNA
 virus is a 3' UTR of a first positive strand single-stranded RNA virus having no DNA
 30 stage.

41. The method of producing a heterologous polypeptide in a transgenic plant of claim 40, wherein the 3' UTR of a first positive strand single-stranded RNA virus having no DNA stage is a 3' UTR of a first bromovirus.

42. The method of producing a heterologous polypeptide in a transgenic plant of claim 41, wherein the 3' UTR of a first bromovirus is a 3' UTR of a first Cowpea chlorotic mottle virus.

43. The method of producing a heterologous polypeptide in a transgenic
5 plant of claim 42, wherein a DNA copy of the 3' UTR of a first Cowpea chlorotic mottle virus comprises the sequence:

AGTGCCCGCTGAAGAGCGTTACACTAGTGTGGCCTACTTGAAGGCTAGTT
ATAACCGTTTCTTTAAACGGTAATCGTTGTTGAAACGTCTTCCTTTTACAA
GAGGATTGAGCTGCCCTTGGGTTTTACTCCTTGAACCCTTCGGAAGAAGCTC
10 TTTGGAGTTCGTACCAGTACCTCACATAGTGAGGTAATAAGACTGGTGGG
CAGCGCCTAGTCGAAAGACTAGGTGATCTCTAAGGAGACC (SEQ ID NO:
8).

44. The method of producing a heterologous polypeptide in a transgenic plant of claim 30, further comprising a sequence complementary to an intron.

15 45. The method of producing a heterologous polypeptide in a transgenic plant of claim 30, further comprising a transcription termination signal.

46. The method of producing a heterologous polypeptide in a transgenic plant of claim 30, wherein the plant is a dicotyledonous plant.

47. The method of producing a heterologous polypeptide in a transgenic
20 plant of claim 46, wherein the dicotyledonous plant is a *Nicotiana* plant.

48. The method of producing a heterologous polypeptide in a transgenic plant of claim 47, wherein the *Nicotiana* plant is a *Nicotiana benthamiana* plant.

49. The method of producing a heterologous polypeptide in a transgenic plant of claim 30, wherein the providing a stimulus to the transgenic plant for
25 synthesis of an RNA complementary to an RNA transcript of the recombinant DNA comprises infecting the transgenic plant with a second positive strand single-stranded RNA virus.

50. The method of producing a heterologous polypeptide in a transgenic plant of claim 49, wherein the second positive strand single-stranded RNA virus is a
30 positive strand single-stranded RNA virus having no DNA stage.

51. The method of producing a heterologous polypeptide in a transgenic plant of claim 50, wherein the second positive strand single-stranded RNA virus having no DNA stage is selected from the group consisting of a positive strand single-

stranded RNA plant virus having no DNA stage and a positive single-stranded RNA animal virus having no DNA stage.

52. The method of producing a heterologous polypeptide in a transgenic plant of claim 51, wherein the second positive strand single-stranded RNA plant virus having no DNA stage is selected from the group consisting of a second Bromovirus, a Tobacco etch virus, a Tobacco vein mottle virus, and a Pepper mottle virus.

53. The method of producing a heterologous polypeptide in a transgenic plant of claim 52, wherein the second Bromovirus is selected from a second Cowpea chlorotic mottle virus and a second Brome mosaic virus.

54. The method of producing a heterologous polypeptide in a transgenic plant of claim 53, wherein the second Bromovirus is a second Cowpea chlorotic mottle virus.

55. The method of producing a heterologous polypeptide in a transgenic plant of claim 30, wherein the providing a stimulus to the cell for synthesis of an RNA complementary to an RNA transcript of the recombinant DNA comprises transfecting the transgenic plant with a cDNA of a second positive strand single-stranded RNA virus.

56. The method of producing a heterologous polypeptide in a transgenic plant of claim 55, wherein the cDNA of a second positive strand single-stranded RNA virus comprises a cDNA encoding an RNA dependent RNA polymerase.

57. The method of producing a heterologous polypeptide in a transgenic plant of claim 56, wherein the second positive strand single-stranded RNA virus is a positive strand single-stranded RNA virus having no DNA stage.

58. The method of producing a heterologous polypeptide in a transgenic plant of claim 57, wherein the second positive strand single-stranded RNA virus having no DNA stage is selected from the group consisting of a positive strand single-stranded RNA plant virus having no DNA stage and a positive single-stranded RNA animal virus having no DNA stage.

59. The method of producing a heterologous polypeptide in a transgenic plant of claim 58, wherein the second positive strand single-stranded RNA plant virus having no DNA stage is selected from the group consisting of a second Bromovirus, a Tobacco etch virus, a Tobacco vein mottle virus, and a Pepper mottle virus.

60. The method of producing a heterologous polypeptide in a transgenic plant of claim 59, wherein the second positive strand single-stranded RNA plant virus

having no DNA stage is selected from the group consisting of a second Cowpea chlorotic mottle virus, a second Brome mosaic virus, a second Tobacco etch virus, a second Tobacco vein mottle virus, and a second Pepper mottle virus.

61. The method of producing a heterologous polypeptide in a transgenic
5 plant of claim 60, wherein the second Bromovirus is selected from a second Cowpea chlorotic mottle virus and a Brome mosaic virus.

62. The method of producing a heterologous polypeptide in a transgenic
plant of claim 30, wherein the providing a stimulus to the cell for synthesis of an
RNA complementary to an RNA transcript of the recombinant DNA comprises
10 transfecting the transgenic plant with RNA of a second positive strand single-stranded
RNA virus, the RNA comprising at least one sequence encoding a polypeptide
component of an RNA virus replication complex.

63. The method of producing a heterologous polypeptide in a transgenic
plant of claim 62, wherein the RNA comprising at least one sequence encoding a
15 polypeptide component of an RNA virus replication complex is an RNA comprising a
sequence encoding an RNA-dependent RNA polymerase.

64. The method of producing a heterologous polypeptide in a transgenic
plant of claim 63, wherein the second positive strand single-stranded RNA virus is a
positive strand single-stranded RNA virus having no DNA stage.

20 65. The method of producing a heterologous polypeptide in a transgenic
plant of claim 64, wherein the second positive strand single-stranded RNA virus
having no DNA stage is selected from the group consisting of a positive strand single-
stranded RNA plant virus having no DNA stage and a positive single-stranded RNA
animal virus having no DNA stage.

25 66. The method of producing a heterologous polypeptide in a transgenic
plant of claim 65, wherein the second positive strand single-stranded RNA plant virus
having no DNA stage is selected from the group consisting of a second Bromovirus, a
Tobacco etch virus, a Tobacco vein mottle virus, and a Pepper mottle virus.

67. The method of producing a heterologous polypeptide in a transgenic
30 plant of claim 66, wherein the second Bromovirus is selected from a second Cowpea
chlorotic mottle virus and a second Brome mosaic virus.

68. The method of producing a heterologous polypeptide in a transgenic
plant of claim 67, wherein the second Bromovirus is a second Cowpea chlorotic
mottle virus.

69. The method of producing a heterologous polypeptide in a transgenic plant of claim 30, wherein the molar concentration ratio of heterologous polypeptide in a cell provided a stimulus relative to a cell not provided a stimulus is at least about 50:1.

5 70. The method of producing a heterologous polypeptide in a transgenic plant of claim 69, wherein the molar concentration ratio of heterologous polypeptide in a cell provided a stimulus relative to a cell not provided a stimulus is at least about 100:1.

10 71. The method of producing a heterologous polypeptide in a transgenic plant of claim 70, wherein the molar concentration ratio of heterologous polypeptide in a cell provided a stimulus relative to a cell not provided a stimulus is at least about 1000:1.

15 72. The method of producing a heterologous polypeptide in a transgenic plant of claim 71, wherein the molar concentration ratio of heterologous polypeptide in a cell provided a stimulus relative to a cell not provided a stimulus is at least about 10,000:1.

73. A method of producing a heterologous polypeptide in a transgenic cell, the method comprising:

20 a) providing a cell comprising a recombinant DNA molecule comprising a promoter operably linked to a DNA sequence comprising, in the 5' to 3' direction,

 i) a sequence complementary to a coding sequence for a heterologous polypeptide;

25 ii) a sequence complementary to an internal ribosome entry site;

 iii) a 3' UTR of a first positive strand single-stranded RNA virus; and

 b) providing a stimulus to the cell for synthesis of an RNA complementary to an RNA transcript of the recombinant DNA.

30 74. The method of producing a heterologous polypeptide in a transgenic cell of claim 73, wherein the promoter is selected from the group consisting of a constitutive promoter and an inducible promoter.

75. The method of producing a heterologous polypeptide in a transgenic cell of claim 74, wherein the promoter is a constitutive promoter.

[0070] This example illustrates plasmid construction of vectors, including plasmids comprising part of a CCMV RNA3 gene 3a open reading frame (ORF) plus all or part of a CCMV RNA3 3' UTR.

[0071] Wild type CCMV RNA3 has a 3a gene ORF and a coat protein (CP) ORF (figure 2). A cDNA copy is maintained in plasmid pCC3TP4. A *Not I* restriction site was introduced near the 3' end of the CP gene ORF in plasmid pCC3AG1 as well as the viral transgenes in transgenic plants 3-57 and $\Delta 69$ (Greene and Allison, *Science* 263: 1423-1425, 1994; Greene and Allison, *Virology* 225: 231-234, 1996). Transgenic plant 3-57 was transformed with the 3' 2/3 of the CP ORF and the full-length 3' UTR. Transgenic plant $\Delta 69$ was transformed with the same viral gene, but the 3' UTR bears a 69-nucleotide deletion at the 3' end. The negative sense RNA-specific primer RA83 (5'-AAGTGGATCCCCTC TTGTGCGGCTGC-3' (SEQ ID NO: 1)) anneals at nucleotides 1519-1544, and was used for first strand cDNA synthesis and PCR. An additional primer RA84 (5'-ACTCCAAAGAGTTCTTCCG-3' (SEQ ID NO: 2)) anneals at nucleotides 2072-2090, and was used for PCR.

[0072] Example 3

[0073] This example illustrates synthesis of a complementary copy of a viral transgene during viral replication, as well as detection of synthesis of a complementary copy of a viral transgene during viral replication.

[0074] A study was undertaken to determine if infection of a transgenic plant with either a wild type bromé mosaic bromovirus (BMV) or a CCMV leads to synthesis of a complementary copy of a transcript of a viral transgene.

[0075] Three sets of plant materials were used in the study: nontransgenic *Nicotiana benthamiana*, clonally propagated transgenic *N. benthamiana* strain 3-57 and clonally propagated transgenic *N. benthamiana* strain $\Delta 69$. Strain 3-57 comprises a 694 nucleotide CCMV transgene comprising 451 3' nucleotides of the viral coat gene and a complete 243 nucleotide CCMV 3' UTR that is naturally contiguous with the viral coat protein gene (Greene and Allison, *Science* 263: 1423-1425, 1994). Transgenic strain $\Delta 69$ is similar but except that the terminal 69 nucleotides of the 3' UTR are deleted. Transgenic transcripts comprising a fragment of the transgenic coat gene used in both transgenic strains were distinguishable from wild type viral transcripts comprising coat gene by the alteration of nucleotides near the 3' end of the coat gene to create a *Not I* restriction site in each transgene (Greene and Allison,

example, incorporate coding sequence for a heterologous polypeptide into the DNA vector such that transcription of the vector would yield a transcript comprising, in the 5' to 3' direction, the complement of the coding sequence, the complement of the IRES, and the 3' UTR. The kit can further comprise a positive strand single-stranded RNA virus or nucleic acid thereof that, upon infection or transfection, would support the formation of an RNA complementary to the recombinant RNA. The kit can further comprise a host organism for growing the vector, such as, for example, transformation-competent *E. coli*. In some aspects, the kit can further comprise laboratory disposables such as, for example, plastic tubes and pipette tips. The kit can further comprise instructions and packaging.

EXAMPLES

[0066] Example 1

[0067] This example illustrates recombination of complementary copies of viral transgenes during viral replication.

[0068] Cowpea chlorotic mottle bromovirus (CCMV) was used initially to demonstrate that transgenic viral gene transcripts are available in the cytoplasm for recombination with a replicating virus (Greene and Allison, *Science* 263: 1423-1425, 1994). In these experiments, transgenic transcripts included part of the viral coat gene as well as a complete CCMV 3' UTR. However, when a portion or all of the 3' UTR was deleted from the transgenic viral gene transcript, viral recombination was below detection limits, suggesting that recombination of a transcript of a viral transgene requires the presence of an intact 3' UTR in the transcript. Without being limited by theory, the observations suggested that the presence of a complete 3' UTR enhances the stability of a transcript of the transgene in the cytoplasm, thereby prolonging the transcript's availability for recombination. These observations raise the possibility that the complete 3' UTR and its replication complex binding site may be recognized by a replication complex of a challenging virus, and a complementary copy of a transgenic transcript capable of contributing to recombination events may be synthesized in the cytoplasm. Recombination events could involve both an original transgenic transcript and its complementary copy, and could occur during either positive or negative strand synthesis.

[0069] Example 2

76. The method of producing a heterologous polypeptide in a transgenic cell of claim 75, wherein the constitutive promoter is a eukaryotic constitutive promoter selected from the group consisting of a cauliflower mosaic virus 35S promoter, a blueberry red ringspot virus promoter, a ubiquitin gene promoter, an actin gene promoter, an NeIF-4A10 promoter, a maize Adh1-based pEmu promoter, a barley leaf thionin BTH6 promoter, a cassava vein mosaic virus promoter, a sugarcane bacilliform badnavirus promoter and a histone gene promoter.

77. The method of producing a heterologous polypeptide in a transgenic cell of claim 76, wherein the eukaryotic constitutive promoter is a cauliflower mosaic virus 35S promoter.

78. The method of producing a heterologous polypeptide in a transgenic plant of claim 77, wherein the cauliflower mosaic virus 35S promoter comprises the sequence:

AGATTAGCCTTTTCAATTTTCAGAAAGAATGCTAACCCACAGATGGTTAGA
GAGGCTTACGCAGCAGGTCTCATCAAGACGATCTACCCGAGCAATAATCT
CCAGGAAATCAAATACCTTCCCAAGAAGGTTAAAGATGCAGTCAAAAGAT
TCAGGACTAACTGCATCAAGAACACAGAGAAAGATATATTTCTCAAGATC
AGAAGTACTATTCCAGTATGGACGATTCAAGGCTTGCTTCACAAACCAAG
GCAAGTAATAGAGATTGGAGTCTCTAAAAAGGTAGTTCCCACTGAATCAA
AGGCCATGGAGTCAAAGATTCAAATAGAGGACCTAACAGAACTCGCCGTA
AAGACTGGCGAACAGTTCATACAGAGTCTCTTACGACTCAATGACAAGAA
GAAAATCTTCGTCAACATGGTGGAGCACGACACACTTGTCTACTCCAAAA
ATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTGAGACTTTTCAA
CAAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGT
CACTTTATTGTGAAGATAGTGGAAAAGGAAGGTGGCTCCTACAAATGCCA
TCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTG
GTCCCAAAGATGGACCCCCACCCACGAGGAGCATCGTGGAAGAAAGAAGA
CGTTCCAACCACGTCTTCAAAGCAAGTGGATTGATGTGATATCTCCACTGA
CGTAAGGGATGACGCACAATCCCACTATCCTTCGCAAGACCCTTCCTCTAT
ATAAGGAAGTTCATTTCAATTTGGAGAGAACACG (SEQ ID NO: 3).

79. The method of producing a heterologous polypeptide in a transgenic cell of claim 73, wherein the coding sequence for a heterologous polypeptide encodes a polypeptide selected from the group consisting of a hormone, an enzyme, a cell toxin, a viral polypeptide, a cell surface polypeptide, and an intracellular polypeptide.

80. The method of producing a heterologous polypeptide in a transgenic cell of claim 73, wherein the sequence complementary to an IRES is a sequence complementary to an IRES selected from the group consisting of a picornavirus IRES, a foot-and-mouth disease virus IRES, an encephalomyocarditis virus IRES, a hepatitis A virus IRES, a hepatitis C virus IRES, a human rhinovirus IRES, a poliovirus IRES, a swine vesicular disease virus IRES, a turnip mosaic potyvirus IRES, a human fibroblast growth factor 2 mRNA IRES, a pestivirus IRES, a Leishmania RNA virus IRES, a Moloney murine leukemia virus IRES, a human rhinovirus 14 IRES, an aphthovirus IRES, a human immunoglobulin heavy chain binding protein mRNA IRES, a *Drosophila* Antennapedia mRNA IRES, a human fibroblast growth factor 2 mRNA IRES, a hepatitis G virus IRES, a tobamovirus IRES, a vascular endothelial growth factor mRNA IRES, a Cocksackie B group virus IRES, a c-myc protooncogene mRNA IRES, a human MYT2 mRNA IRES, a human parechovirus type 1 virus IRES, a human parechovirus type 2 virus IRES, a eukaryotic initiation factor 4GI mRNA IRES, a *Plautia stali* intestine virus IRES, a Theiler's murine encephalomyelitis virus IRES, a bovine enterovirus IRES, a connexin 43 mRNA IRES, a homeodomain protein Gtx mRNA IRES, an AML1 transcription factor mRNA IRES, an NF-kappa B repressing factor mRNA IRES, an X-linked inhibitor of apoptosis mRNA IRES, a cricket paralysis virus RNA IRES, a p58(PITSLRE) protein kinase mRNA IRES, an ornithine decarboxylase mRNA IRES, a connexin-32 mRNA IRES, a bovine viral diarrhea virus IRES, an insulin-like growth factor I receptor mRNA IRES, a human immunodeficiency virus type 1 gag gene IRES, a classical swine fever virus IRES, a Kaposi's sarcoma-associated herpes virus IRES, a short IRES selected from a library of random oligonucleotides, a Jembrana disease virus IRES, an apoptotic protease-activating factor 1 mRNA IRES, a *Rhopalosiphum padi* virus IRES, a cationic amino acid transporter mRNA IRES, a human insulin-like growth factor II leader 2 mRNA IRES, a giardiavirus IRES, a Smad5 mRNA IRES, a porcine teschovirus-1 talfan IRES, a *Drosophila* Hairless mRNA IRES, an hSNM1 mRNA IRES, a Cbfa1/Runx2 mRNA IRES, an Epstein-Barr virus IRES, a hibiscus chlorotic ringspot virus IRES, a rat pituitary vasopressin V1b receptor mRNA IRES, and a human hsp70 mRNA IRES.

81. The method of producing a heterologous polypeptide in a transgenic cell of claim 80, wherein the sequence complementary to an internal ribosome entry site is a sequence complementary to a picornavirus internal ribosome entry site.

82. The method of producing a heterologous polypeptide in a transgenic cell of claim 81, wherein the sequence complementary to a picornavirus internal ribosome entry site comprises the sequence:

TTATCATCGTGTTTTTCAAAGGAAAACACGTCCCCGTGGTTCGGGGGGGCC
5 TAGACGTTTTTTTAACTCGACTAAACACATGTAAAGCATGTGCACCGAG
GCCCCAGATCAGATCCCATAACAATGGGGTACCTTCTGGGCATCCTTCAGCC
CCTTGTTGAATACGCTTGAGGAGAGCCATTTGACTCTTCCACAACACTATCC
AACTCACAACGTGGCACTGGGGTTGTGCCGCCTTGCAGGTGTATCTTATA
CACGTGGCTTTTGGCCGCAGAGGCACCTGTCGCCAGGTGGGGGGTTCCGC
10 TGCCTGCAAAGGGTCGCTACAGACGTTGTTTGTCTTCAAGAAGCTTCCAGA
GGAAGTCTTCCTTCACGACATTCAACAGACCTTGCATTCCTTTGGCGAGA
GGGGAAAGACCCCTAGGAATGCTCGTCAAGAAGACAGGGCCAGGTTTCC
GGGCCCTCACATTGCCAAAAGACGGCAATATGGTGGAAAATCACATATAG
ACAAACGCACACCGGCCTTATTCCAAGCGGCTTCGGCCAGTAACGTTAGG
15 GGGGGGGGAGGGAGAGGGGCGGAATT (SEQ ID NO: 6).

83. The method of producing a heterologous polypeptide in a transgenic cell of claim 73, wherein the 3' UTR of a first positive strand single-stranded RNA virus is a 3' UTR of a first positive strand single-stranded RNA virus having no DNA stage.

20 84. The method of producing a heterologous polypeptide in a transgenic cell of claim 83, wherein the 3' UTR of a first positive strand single-stranded RNA virus having no DNA stage is a 3' UTR of a first bromovirus.

85. The method of producing a heterologous polypeptide in a transgenic cell of claim 84, wherein the 3' UTR of a first bromovirus is a 3' UTR of a first
25 Cowpea chlorotic mottle virus.

86. The method of producing a heterologous polypeptide in a transgenic cell of claim 85, wherein a DNA copy of the 3' UTR of a first Cowpea chlorotic mottle virus comprises the sequence:

AGTGCCCGCTGAAGAGCGTTACACTAGTGTGGCCTACTTGAAGGCTAGTT
30 ATAACCGTTTCTTTAAACGGTAATCGTTGTTGAAACGTCTTCCTTTTACAA
GAGGATTGAGCTGCCCTTGGGTTTTACTCCTTGAACCTTCGGAAGAACTC
TTTGGAGTTCGTACCAGTACCTCACATAGTGAGGTAATAAGACTGGTGGG
CAGCGCCTAGTCGAAAGACTAGGTGATCTCTAAGGAGACC (SEQ ID NO:
8).

87. The method of producing a heterologous polypeptide in a transgenic cell of claim 73, further comprising a sequence complementary to an intron.

88. The method of producing a heterologous polypeptide in a transgenic cell of claim 73, further comprising a transcription termination signal.

5 89. The method of producing a heterologous polypeptide in a transgenic cell of claim 73, wherein the recombinant DNA molecule is comprised by a host cell.

90. The method of producing a heterologous polypeptide in a transgenic cell of claim 89, wherein the host cell is a plant cell.

10 91. The method of producing a heterologous polypeptide in a transgenic cell of claim 90, wherein the plant cell is comprised by a plant.

92. The method of producing a heterologous polypeptide in a transgenic cell of claim 91, wherein the plant is a dicotyledonous plant.

93. The method of producing a heterologous polypeptide in a transgenic cell of claim 92, wherein the dicotyledonous plant is a *Nicotiana* plant.

15 94. The method of producing a heterologous polypeptide in a transgenic cell of claim 93, wherein the *Nicotiana* plant is a *Nicotiana benthamiana* plant.

95. The method of producing a heterologous polypeptide in a transgenic cell of claim 73, wherein the providing a stimulus to the cell for synthesis of an RNA complementary to an RNA transcript of the recombinant DNA comprises infecting
20 the transgenic cell with a second positive strand single-stranded RNA virus.

96. The method of producing a heterologous polypeptide in a transgenic cell of claim 95, wherein the second positive strand single-stranded RNA virus is a positive strand single-stranded RNA virus having no DNA stage.

97. The method of producing a heterologous polypeptide in a transgenic
25 cell of claim 96, wherein the second positive strand single-stranded RNA virus having no DNA stage is selected from the group consisting of a positive strand single-stranded RNA plant virus having no DNA stage and a positive single-stranded RNA animal virus having no DNA stage.

98. The method of producing a heterologous polypeptide in a transgenic
30 cell of claim 97, wherein the second positive strand single-stranded RNA plant virus having no DNA stage is selected from the group consisting of a second Bromovirus, a Tobacco etch virus, a Tobacco vein mottle virus, and a Pepper mottle virus.

99. The method of producing a heterologous polypeptide in a transgenic cell of claim 98, wherein the second Bromovirus is selected from a second Cowpea chlorotic mottle virus and a second Brome mosaic virus.

100. The method of producing a heterologous polypeptide in a transgenic
5 cell of claim 99, wherein the second Bromovirus is a second Cowpea chlorotic mottle virus.

101. The method of producing a heterologous polypeptide in a transgenic cell of claim 73, wherein the providing a stimulus to the cell for synthesis of an RNA complementary to an RNA transcript of the recombinant DNA comprises transfecting
10 the transgenic cell with a cDNA of a second positive strand single-stranded RNA virus.

102. The method of producing a heterologous polypeptide in a transgenic cell of claim 101, wherein the cDNA of a second positive strand single-stranded RNA virus comprises a cDNA encoding an RNA dependent RNA polymerase.

15 103. The method of producing a heterologous polypeptide in a transgenic cell of claim 101, wherein the second positive strand single-stranded RNA virus is a positive strand single-stranded RNA virus having no DNA stage.

104. The method of producing a heterologous polypeptide in a transgenic cell of claim 103, wherein the second positive strand single-stranded RNA virus
20 having no DNA stage is selected from the group consisting of a positive strand single-stranded RNA plant virus having no DNA stage and a positive single-stranded RNA animal virus having no DNA stage.

105. The method of producing a heterologous polypeptide in a transgenic cell of claim 104, wherein the second positive strand single-stranded RNA plant virus
25 having no DNA stage is selected from the group consisting of a second Bromovirus, a Tobacco etch virus, a Tobacco vein mottle virus, and a Pepper mottle virus.

106. The method of producing a heterologous polypeptide in a transgenic cell of claim 105, wherein the second positive strand single-stranded RNA plant virus having no DNA stage is selected from the group consisting of a second Cowpea
30 chlorotic mottle virus, a second Brome mosaic virus, a second Tobacco etch virus, a second Tobacco vein mottle virus, and a second Pepper mottle virus.

107. The method of producing a heterologous polypeptide in a transgenic cell of claim 106, wherein the second Bromovirus is selected from a second Cowpea chlorotic mottle virus and a Brome mosaic virus.

108. The method of producing a heterologous polypeptide in a transgenic cell of claim 73, wherein the providing a stimulus to the cell for synthesis of an RNA complementary to an RNA transcript of the recombinant DNA comprises transfecting the transgenic cell with RNA of a second positive strand single-stranded RNA virus,
5 the RNA comprising at least one sequence encoding a polypeptide component of an RNA virus replication complex.

109. The method of producing a heterologous polypeptide in a transgenic cell of claim 108, wherein the RNA comprising at least one sequence encoding a polypeptide component of an RNA virus replication complex is an RNA comprising a
10 sequence encoding an RNA-dependent RNA polymerase.

110. The method of producing a heterologous polypeptide in a transgenic cell of claim 109, wherein the second positive strand single-stranded RNA virus is a positive strand single-stranded RNA virus having no DNA stage.

111. The method of producing a heterologous polypeptide in a transgenic
15 cell of claim 110, wherein the second positive strand single-stranded RNA virus having no DNA stage is selected from the group consisting of a positive strand single-stranded RNA plant virus having no DNA stage and a positive single-stranded RNA animal virus having no DNA stage.

112. The method of producing a heterologous polypeptide in a transgenic
20 cell of claim 111, wherein the second positive strand single-stranded RNA plant virus having no DNA stage is selected from the group consisting of a second Bromovirus, a Tobacco etch virus, a Tobacco vein mottle virus, and a Pepper mottle virus.

113. The method of producing a heterologous polypeptide in a transgenic
25 cell of claim 112, wherein the second Bromovirus is selected from a second Cowpea chlorotic mottle virus and a second Brome mosaic virus.

114. The method of producing a heterologous polypeptide in a transgenic cell of claim 113, wherein the second Bromovirus is a second Cowpea chlorotic mottle virus.

115. The method of producing a heterologous polypeptide in a transgenic
30 cell of claim 73, wherein the molar concentration ratio of heterologous polypeptide in a cell provided a stimulus relative to a cell not provided a stimulus is at least about 50:1.

116. The method of producing a heterologous polypeptide in a transgenic cell of claim 115, wherein the molar concentration ratio of heterologous polypeptide

in a cell provided a stimulus relative to a cell not provided a stimulus is at least about 100:1.

117. The method of producing a heterologous polypeptide in a transgenic cell of claim 116, wherein the molar concentration ratio of heterologous polypeptide in a cell provided a stimulus relative to a cell not provided a stimulus is at least about 1000:1.

118. The method of producing a heterologous polypeptide in a transgenic cell of claim 117, wherein the molar concentration ratio of heterologous polypeptide in a cell provided a stimulus relative to a cell not provided a stimulus is at least about 10,000:1.

119. A recombinant DNA molecule comprising a promoter operably linked to a DNA sequence comprising, in the 5' to 3' direction:

- a) a sequence complementary to a coding sequence for a heterologous polypeptide;
- b) a sequence complementary to an internal ribosome entry site;
- and
- c) a 3' UTR of a positive strand single-stranded RNA virus.

120. The recombinant DNA molecule of claim 119, wherein the promoter is a selected from the group consisting of a constitutive promoter and an inducible promoter.

121. The recombinant DNA molecule of claim 120, wherein the promoter is a constitutive promoter.

122. The recombinant DNA molecule of claim 121, wherein the constitutive promoter is a eukaryotic constitutive promoter selected from the group consisting of a cauliflower mosaic virus 35S promoter, a blueberry red ringspot virus promoter, a ubiquitin gene promoter, an actin gene promoter, an NeIF-4A10 promoter, a maize Adh1-based pEmu promoter, a barley leaf thionin BTH6 promoter, a cassava vein mosaic virus promoter, a sugarcane bacilliform badnavirus promoter and a histone gene promoter.

123. The recombinant DNA molecule of claim 122, wherein the eukaryotic constitutive promoter is a cauliflower mosaic virus 35S promoter.

124. The recombinant DNA molecule of claim 123, wherein the cauliflower mosaic virus 35S promoter comprises the sequence:
AGATTAGCCTTTTCAATTTTCAGAAAGAATGCTAACCCACAGATGGTTAGA

GAGGCTTACGCAGCAGGTCTCATCAAGACGATCTACCCGAGCAATAATCT
 CCAGGAAATCAAATACCTTCCCAAGAAGGTTAAAGATGCAGTCAAAAGAT
 TCAGGACTAACTGCATCAAGAACACAGAGAAAGATATATTTCTCAAGATC
 AGAAGTACTATTCCAGTATGGACGATTCAAGGCTTGCTTCACAAACCAAG
 5 GCAAGTAATAGAGATTGGAGTCTCTAAAAAGGTAGTTCCCACTGAATCAA
 AGGCCATGGAGTCAAAGATTCAAATAGAGGACCTAACAGAACTCGCCGTA
 AAGACTGGCGAACAGTTCATACAGAGTCTCTTACGACTCAATGACAAGAA
 GAAAATCTTCGTCAACATGGTGGAGCACGACACACTTGTCTACTCCAAAA
 ATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTGAGACTTTTCAA
 10 CAAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTGCCAGCTATCTGT
 CACTTTATTGTGAAGATAGTGGAAAAGGAAGGTGGCTCCTACAAATGCCA
 TCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTG
 GTCCCAAAGATGGACCCCCACCCACGAGGAGCATCGTGGAAGAAAGAAGA
 CGTTCCAACCACGTCTTCAAAGCAAGTGGATTGATGTGATATCTCCACTGA
 15 CGTAAGGGATGACGCACAATCCCACTATCCTTCGCAAGACCCTTCCTCTAT
 ATAAGGAAGTTCATTTCAATTTGGAGAGAACACG (SEQ ID NO: 3).

125. The recombinant DNA molecule of claim 119, wherein the coding
 sequence for a heterologous polypeptide encodes a polypeptide selected from the
 group consisting of a hormone, an enzyme, a cell toxin, a viral polypeptide, a cell
 20 surface polypeptide, and an intracellular polypeptide.

126. The recombinant DNA molecule of claim 119, wherein the sequence
 complementary to an internal ribosome entry site is a sequence complementary to an
 IRES selected from the group consisting of a picornavirus IRES, a foot-and-mouth
 disease virus IRES, an encephalomyocarditis virus IRES, a hepatitis A virus IRES, a
 25 hepatitis C virus IRES, a human rhinovirus IRES, a poliovirus IRES, a swine
 vesicular disease virus IRES, a turnip mosaic potyvirus IRES, a human fibroblast
 growth factor 2 mRNA IRES, a pestivirus IRES, a Leishmania RNA virus IRES, a
 Moloney murine leukemia virus IRES a human rhinovirus 14 IRES, an aphthovirus
 IRES, a human immunoglobulin heavy chain binding protein mRNA IRES, a
 30 *Drosophila* Antennapedia mRNA IRES, a human fibroblast growth factor 2 mRNA
 IRES, a hepatitis G virus IRES, a tobamovirus IRES, a vascular endothelial growth
 factor mRNA IRES, a Coxsackie B group virus IRES, a c-myc protooncogene mRNA
 IRES, a human MYT2 mRNA IRES, a human parechovirus type 1 virus IRES, a
 human parechovirus type 2 virus IRES, a eukaryotic initiation factor 4GI mRNA

IRES, a Plautia stali intestine virus IRES, a Theiler's murine encephalomyelitis virus
 IRES, a bovine enterovirus IRES, a connexin 43 mRNA IRES, a homeodomain
 protein Gtx mRNA IRES, an AML1 transcription factor mRNA IRES, an NF-kappa B
 repressing factor mRNA IRES, an X-linked inhibitor of apoptosis mRNA IRES, a
 5 cricket paralysis virus RNA IRES, a p58(PITSLRE) protein kinase mRNA IRES, an
 ornithine decarboxylase mRNA IRES, a connexin-32 mRNA IRES, a bovine viral
 diarrhea virus IRES, an insulin-like growth factor I receptor mRNA IRES, a human
 immunodeficiency virus type 1 gag gene IRES, a classical swine fever virus IRES, a
 Kaposi's sarcoma-associated herpes virus IRES, a short IRES selected from a library
 10 of random oligonucleotides, a Jembrana disease virus IRES, an apoptotic protease-
 activating factor 1 mRNA IRES, a Rhopalosiphum padi virus IRES, a cationic amino
 acid transporter mRNA IRES, a human insulin-like growth factor II leader 2 mRNA
 IRES, a giardavirus IRES, a Smad5 mRNA IRES, a porcine teschovirus-1 talfan
 IRES, a *Drosophila* Hairless mRNA IRES, an hSNM1 mRNA IRES, a Cbfa1/Runx2
 15 mRNA IRES, an Epstein-Barr virus IRES, a hibiscus chlorotic ringspot virus IRES, a
 rat pituitary vasopressin V1b receptor mRNA IRES, and a human hsp70 mRNA
 IRES.

127. The recombinant DNA molecule of claim 126, wherein the sequence
 complementary to an internal ribosome entry site is a sequence complementary to a
 20 picornavirus internal ribosome entry site.

128. The recombinant DNA molecule of claim 127, wherein the sequence
 complementary to a picornavirus internal ribosome entry site comprises the sequence:
 TTATCATCGTGTGTTTTCAAAGGAAAACACGTCCTCCGTTGCGGGGGGCC
 TAGACGTTTTTTTAACCTCGACTAAACACATGTAAAGCATGTGCACCGAG
 25 GCCCCAGATCAGATCCCATAACAATGGGGTACCTTCTGGGCATCCTTCAGCC
 CCTTGTGAATACGCTTGAGGAGAGCCATTTGACTCTTTCCACAACCTATCC
 AACTCACAACGTGGCACTGGGGTTGTGCCGCTTTGCAGGTGTATCTTATA
 CACGTGGCTTTTGGCCGCAGAGGCACCTGTCGCCAGGTGGGGGGTTCCGC
 TGCCTGCAAAGGGTCGCTACAGACGTTGTTTGTCTTCAAGAAGCTTCCAGA
 30 GGAAGTCTTCCTTCACGACATTCAACAGACCTTGCATTCCTTTGGCGAGA
 GGGGAAAGACCCCTAGGAATGCTCGTCAAGAAGACAGGGCCAGGTTTCC
 GGGCCCTCACATTGCCAAAAGACGGCAATATGGTGGAAAATCACATATAG
 ACAAACGCACACCGGCCTTATTCCAAGCGGCTTCGGCCAGTAACGTTAGG
 GGGGGGGGAGGGAGAGGGGCGGAATT (SEQ ID NO: 6).

129. The recombinant DNA molecule of claim 119, wherein the 3' UTR of a positive strand single-stranded RNA virus is a 3' UTR of a positive strand single-stranded RNA virus having no DNA stage.

130. The recombinant DNA molecule of claim 129, wherein the 3' UTR of a positive strand single-stranded RNA virus having no DNA stage is a 3' UTR of a bromovirus.

131. The recombinant DNA molecule of claim 130, wherein the 3' UTR of a bromovirus is a 3' UTR of a Cowpea chlorotic mottle virus.

132. The recombinant DNA molecule of claim 131, wherein a DNA copy of the 3' UTR of a Cowpea chlorotic mottle virus comprises the sequence:
AGTGCCCGCTGAAGAGCGTTACACTAGTGTGGCCTACTTGAAGGCTAGTT
ATAACCGTTTCTTTAAACGGTAATCGTTGTTGAAACGTCTTCCTTTTACAA
GAGGATTGAGCTGCCCTTGGGTTTACTCCTTGAACCCTTCGGAAGAAGCTC
TTTGGAGTTCGTACCAGTACCTCACATAGTGAGGTAATAAGACTGGTGGG
CAGCGCCTAGTCGAAAGACTAGGTGATCTCTAAGGAGACC (SEQ ID NO: 8).

133. The recombinant DNA molecule of claim 119, further comprising a sequence complementary to an intron.

134. The recombinant DNA molecule of claim 119, further comprising a transcription termination signal.

135. A transgenic host cell comprising the recombinant DNA molecule of claim 119.

136. The transgenic host cell of claim 134, wherein the transgenic host cell is a transgenic plant cell.

137. A transgenic plant comprising the transgenic plant cell of claim 136.

138. The transgenic plant of claim 137, wherein the transgenic plant is a transgenic dicotyledonous plant.

139. The transgenic dicotyledonous plant of claim 138, wherein the transgenic dicotyledonous plant is a transgenic *Nicotiana* plant.

140. The transgenic *Nicotiana* plant of claim 139, wherein the transgenic *Nicotiana* plant is a transgenic *Nicotiana benthamiana* plant.

141. Transgenic seed comprising the recombinant DNA molecule of claim 119.

142. A recombinant RNA molecule comprising, in the 5' to 3' direction:

a) an RNA sequence comprising a sequence complementary to a coding sequence for a heterologous polypeptide;

b) a sequence complementary to an internal ribosome entry site; and

5 c) a 3' UTR of a positive strand single-stranded RNA virus.

143. The recombinant RNA molecule of claim 142, wherein the coding sequence for a heterologous polypeptide encodes a polypeptide selected from the group consisting of a hormone, an enzyme, a cell toxin, a viral polypeptide, a cell surface polypeptide, and an intracellular polypeptide.

10 144. The recombinant RNA molecule of claim 142, wherein the sequence complementary to an internal ribosome entry site is a sequence complementary to an IRES selected from the group consisting of a picornavirus IRES, a foot-and-mouth disease virus IRES, an encephalomyocarditis virus IRES, a hepatitis A virus IRES, a hepatitis C virus IRES, a human rhinovirus IRES, a poliovirus IRES, a swine
15 vesicular disease virus IRES, a turnip mosaic potyvirus IRES, a human fibroblast growth factor 2 mRNA IRES, a pestivirus IRES, a Leishmania RNA virus IRES, a Moloney murine leukemia virus IRES, a human rhinovirus 14 IRES, an aphthovirus IRES, a human immunoglobulin heavy chain binding protein mRNA IRES, a *Drosophila* Antennapedia mRNA IRES, a human fibroblast growth factor 2 mRNA
20 IRES, a hepatitis G virus IRES, a tobamovirus IRES, a vascular endothelial growth factor mRNA IRES, a Coxsackie B group virus IRES, a c-myc protooncogene mRNA IRES, a human MYT2 mRNA IRES, a human parechovirus type 1 virus IRES, a human parechovirus type 2 virus IRES, a eukaryotic initiation factor 4GI mRNA IRES, a *Plautia stali* intestine virus IRES, a Theiler's murine encephalomyelitis virus
25 IRES, a bovine enterovirus IRES, a connexin 43 mRNA IRES, a homeodomain protein Gtx mRNA IRES, an AML1 transcription factor mRNA IRES, an NF-kappa B repressing factor mRNA IRES, an X-linked inhibitor of apoptosis mRNA IRES, a cricket paralysis virus RNA IRES, a p58(PITSLRE) protein kinase mRNA IRES, an ornithine decarboxylase mRNA IRES, a connexin-32 mRNA IRES, a bovine viral
30 diarrhea virus IRES, an insulin-like growth factor I receptor mRNA IRES, a human immunodeficiency virus type 1 gag gene IRES, a classical swine fever virus IRES, a Kaposi's sarcoma-associated herpes virus IRES, a short IRES selected from a library of random oligonucleotides, a Jembrana disease virus IRES, an apoptotic protease-activating factor 1 mRNA IRES, a Rhopalosiphum padi virus IRES, a cationic amino

acid transporter mRNA IRES, a human insulin-like growth factor II leader 2 mRNA IRES, a giardiavirus IRES, a Smad5 mRNA IRES, a porcine teschovirus-1 talfan IRES, a *Drosophila* Hairless mRNA IRES, an hSNM1 mRNA IRES, a Cbfa1/Runx2 mRNA IRES, an Epstein-Barr virus IRES, a hibiscus chlorotic ringspot virus IRES, a
 5 rat pituitary vasopressin V1b receptor mRNA IRES, and a human hsp70 mRNA IRES.

145. The recombinant RNA molecule of claim 144, wherein the sequence complementary to an internal ribosome entry site is a sequence complementary to a
 10 picornavirus internal ribosome entry site.

146. The recombinant RNA molecule of claim 145, wherein the sequence complementary to a picornavirus internal ribosome entry site comprises the sequence:
 UUAUCAUCGUGUUUUUCAAGGAAAACACGUCCTCCGUGGUUCGGGGG
 GCCUAGACGUUUUUUAACCUCGACUAAACACAUGUAAAGCAUGUGCA
 CCGAGGCCCCAGAUCAUAUCCCAUACAAUGGGGUACCUUCUGGGCAUCC
 15 UUCAGCCCCUUGUUGAAUACGCUUGAGGAGAGCCAUUUGACUCUUUCC
 ACAACUAUCCAACUCACAACGUGGGCACUGGGGUUGUGCCGCCUUUGCAG
 GUGUAUCUUAUACACGUGGCUUUUGGCCGCAGAGGCACCUGUCGCCAG
 GUGGGGGGUUCCGCUGCCUGCAAAGGGUUCGCUACAGACGUUGUUUGUC
 UUCAAGAAGCUUCCAGAGGAACUGCUUCCUUCACGACAUAUCAAACAGACC
 20 UUGCAUUCCUUUGGCGAGAGGGGAAAGACCCCUAGGAAUGCUCGUCAA
 GAAGACAGGGCCAGGUUUCCGGGCCCUCACAUUGCCAAAAGACGGCAAU
 AUGGUGGAAAAUCACAUUAUAGACAAACGCACACCGGCCUUAUCCAAG
 CGGCUUCGGCCAGUAACGUUAGGGGGGGGGGAGGGAGAGGGGGCGGAAU
 U (SEQ ID NO: 7).

25 147. The recombinant RNA molecule of claim 142, wherein the 3' UTR of a positive strand single-stranded RNA virus is a 3' UTR of a positive strand single-stranded RNA virus having no DNA stage.

148. The recombinant RNA molecule of claim 147, wherein the 3' UTR of a positive strand single-stranded RNA virus having no DNA stage is a 3' UTR of a
 30 bromovirus

149. The recombinant RNA molecule of claim 148, wherein the 3' UTR of a bromovirus is a 3' UTR of a Cowpea chlorotic mottle virus.

150. The recombinant RNA molecule of claim 149, wherein an RNA copy of the 3' UTR of a Cowpea chlorotic mottle virus comprises the sequence:

AGUGCCCGCUGAAGAGCGUUACACUAGUGUGGCCUACUUGAAGGCUAG
UUAUAACCGUUUCUUUAAACGGUAAUCGUUGUUGAAACGUCUCCUUU
UACAAGAGGAUUGAGCUGCCCUUGGGUUUUACUCCUUGAACCCUUCGG
AAGAACUCUUUGGAGUUCGUACCAGUACCUCACAUAGUGAGGUAAUAA
5 GACUGGUGGGCAGCGCCUAGUCGAAAGACUAGGUGAUCUCUAAGGAGA
CC (SEQ ID NO: 9).

151. The recombinant RNA molecule of claim 142, further comprising a sequence complementary to an intron.

152. A transgenic host cell comprising the recombinant RNA molecule of
10 claim 142.

153. The transgenic host cell of claim 152, wherein the transgenic host cell is a transgenic plant cell.

154. A transgenic plant comprising the transgenic plant cell of claim 153.

155. The transgenic plant of claim 154, wherein the transgenic plant is a
15 transgenic dicotyledonous plant.

156. The transgenic dicotyledonous plant 155, wherein the transgenic dicotyledonous plant is a transgenic *Nicotiana* plant.

157. The transgenic *Nicotiana* plant of claim 155, wherein the transgenic *Nicotiana* plant is a transgenic *Nicotiana benthamiana* plant.

20 158. Transgenic seed comprising the recombinant RNA of claim 142.

159. An RNA complement of a recombinant RNA molecule, the complement comprising, in the 5' to 3' direction:

- a) a sequence complementary to a 3' UTR of a positive strand single-stranded RNA virus;
- 25 b) an internal ribosome entry site; and
- c) an RNA sequence encoding a heterologous polypeptide.

160. The RNA complement of a recombinant RNA molecule of claim 159, wherein the RNA sequence encoding a heterologous polypeptide encodes a polypeptide selected from the group consisting of a hormone, an enzyme, a cell toxin,
30 a viral polypeptide, a cell surface polypeptide, and an intracellular polypeptide.

161. The RNA complement of a recombinant RNA molecule of claim 159, wherein the internal ribosome entry site is selected from the group consisting of a picornavirus IRES, a foot-and-mouth disease virus IRES, an encephalomyocarditis virus IRES, a hepatitis A virus IRES, a hepatitis C virus IRES, a human rhinovirus

IRES, a poliovirus IRES, a swine vesicular disease virus IRES, a turnip mosaic
 potyvirus IRES, a human fibroblast growth factor 2 mRNA IRES, a pestivirus IRES, a
 Leishmania RNA virus IRES, a Moloney murine leukemia virus IRES a human
 rhinovirus 14 IRES, an aphthovirus IRES, a human immunoglobulin heavy chain
 5 binding protein mRNA IRES, a *Drosophila* Antennapedia mRNA IRES, a human
 fibroblast growth factor 2 mRNA IRES, a hepatitis G virus IRES, a tobamovirus
 IRES, a vascular endothelial growth factor mRNA IRES, a Cocksackie B group virus
 IRES, a c-myc protooncogene mRNA IRES, a human MYT2 mRNA IRES, a human
 parechovirus type 1 virus IRES, a human parechovirus type 2 virus IRES, a
 10 eukaryotic initiation factor 4GI mRNA IRES, a Plautia stali intestine virus IRES, a
 Theiler's murine encephalomyelitis virus IRES, a bovine enterovirus IRES, a
 connexin 43 mRNA IRES, a homeodomain protein Gtx mRNA IRES, an AML1
 transcription factor mRNA IRES, an NF-kappa B repressing factor mRNA IRES, an
 X-linked inhibitor of apoptosis mRNA IRES, a cricket paralysis virus RNA IRES, a
 15 p58(PITSLRE) protein kinase mRNA IRES, an ornithine decarboxylase mRNA
 IRES, a connexin-32 mRNA IRES, a bovine viral diarrhea virus IRES, an insulin-like
 growth factor I receptor mRNA IRES, a human immunodeficiency virus type 1 gag
 gene IRES, a classical swine fever virus IRES, a Kaposi's sarcoma-associated herpes
 virus IRES, a short IRES selected from a library of random oligonucleotides, a
 20 Jembrana disease virus IRES, an apoptotic protease-activating factor 1 mRNA IRES,
 a Rhopalosiphum padi virus IRES, a cationic amino acid transporter mRNA IRES, a
 human insulin-like growth factor II leader 2 mRNA IRES, a giardiavirus IRES, a
 Smad5 mRNA IRES, a porcine teschovirus-1 talfan IRES, a *Drosophila* Hairless
 mRNA IRES, an hSNM1 mRNA IRES, a Cbfa1/Runx2 mRNA IRES, an Epstein-
 25 Barr virus IRES, a hibiscus chlorotic ringspot virus IRES, a rat pituitary vasopressin
 V1b receptor mRNA IRES, and a human hsp70 mRNA IRES.

162. The RNA complement of a recombinant RNA molecule of claim 161, wherein the internal ribosome entry site is a picornavirus internal ribosome entry site.

163. The RNA complement of a recombinant RNA molecule of claim 162,
30 wherein the picornavirus internal ribosome entry site comprises the sequence:
AAUUCCGCCCCUCUCCCUCCCCCCCCCCCUAACGUUACUGGCCGAAGCCGC
UUGGAAUAAGGCCGGUGUGCGUUUGUCUAUAUGUGAUUUUCCACCAUA
UUGCCGUCUUUUGGCAAUGUGAGGGCCCGGAAACCUGGCCCUUGUCUUCU
UGACGAGCAUUCUAGGGGUCUUUCCCCUCUCGCCAAAGGAAUGCAAGG

UCUGUUGAAUGUCGUGAAGGAAGCAGUUCCUCUGGAAGCUUCUUGAAG
ACAAACAACGUCUGUAGCGACCCUUGCAGGCAGCGGAACCCCCCACC
GGCGACAGGUGCCUCUGCGGCCAAAAGCCACGUGUAUAAGAUACACCUG
CAAAGGCGGCACAACCCCAGUGCCACGUUGUGAGUUGGAUAGUUGUGG
5 AAAGAGUCAAAUGGCUCUCCUCAAGCGUAUUAACAAGGGGCUGAAGG
AUGCCCAGAAGGUACCCCAUUGUAUGGGAUCUGAUCUGGGGCCUCGGU
GCACAUGC UUUACAUGUGUUUAGUCGAGGUUAAAAAACGUCUAGGCC
CCCCGAACCACGGGGACGUGGUUUUCCUUGAAAAACACGAUGAUAA
(SEQ ID NO: 5).

10 164. The RNA complement of a recombinant RNA molecule of claim 159,
wherein the complement of a 3' UTR of a positive strand single-stranded RNA virus is
a complement of a 3' UTR of a positive strand single-stranded RNA virus having no
DNA stage.

15 165. The RNA complement of a recombinant RNA molecule of claim 164,
wherein the complement of a 3' UTR of a positive strand single-stranded RNA virus
having no DNA stage is a complement 3' UTR of a bromovirus

166. The RNA complement of a recombinant RNA molecule of claim 165,
wherein the complement of a 3' UTR of a bromovirus is a complement of a 3' UTR of
a Cowpea chlorotic mottle virus.

20 167. The RNA complement of a recombinant RNA molecule of claim 166,
wherein the complement of a 3' UTR of a Cowpea chlorotic mottle virus comprises
the sequence:

GGUCUCCUAGAGAUCACCUAGUCUUUCGACUAGGCGCUGCCCACCAGU
CUUAUUACCUCACUAUGUGAGGUACUGGUACGAACUCCAAAGAGUUCU
25 UCCGAAGGGUUAAGGAGUAAAACCCAAGGGCAGCUCAAUCCUCUUGU
AAAAGGAAGACGUUUAACAACGAUUACCGUUUAAAGAAACGGUUAUA
ACUAGCCUUAAGUAGGCCACACUAGUGUAACGCUCUUCAGCGGGCACU
(SEQ ID NO: 11).

30 168. The RNA complement of a recombinant RNA molecule of claim 159,
further comprising an intron.

169. A transgenic host cell comprising the RNA complement of a
recombinant RNA molecule of claim 159.

170. The transgenic host cell of claim 169, wherein the transgenic host cell
is a transgenic plant cell.

171. A transgenic plant comprising the transgenic plant cell of claim 170.

172. The transgenic plant of claim 171, wherein the transgenic plant is a transgenic dicotyledonous plant.

173. The transgenic dicotyledonous plant of claim 172, wherein the
5 transgenic dicotyledonous plant is a transgenic *Nicotiana* plant.

174. The transgenic *Nicotiana* plant of claim 173, wherein the transgenic *Nicotiana* plant is a transgenic *Nicotiana benthamiana* plant.

175. Transgenic seed comprising the RNA complement of a recombinant RNA molecule of claim 159.

10 176. A recombinant DNA molecule for construction of a vector for expressing a heterologous polypeptide in a transgenic cell, the recombinant DNA molecule comprising a promoter operably linked, in the 5' to 3' direction, to DNA sequence comprising:

a) at least one site for insertion of a sequence comprising coding
15 sequence of a heterologous polypeptide in an antisense orientation;

b) a sequence complementary to an internal ribosome entry site;
and

c) a 3' UTR of a positive strand single-stranded RNA virus.

177. The recombinant DNA molecule for construction of a vector for
20 expressing a heterologous polypeptide in a transgenic cell of claim 176, wherein the promoter is selected from the group consisting of a constitutive promoter and an inducible promoter.

178. The recombinant DNA molecule for construction of a vector for
25 expressing a heterologous polypeptide in a transgenic cell of claim 177, wherein the promoter is a constitutive promoter.

179. The recombinant DNA molecule for construction of a vector for
expressing a heterologous polypeptide in a transgenic cell of claim 178, wherein the
constitutive promoter is a eukaryotic constitutive promoter selected from the group
consisting of a cauliflower mosaic virus 35S promoter, a blueberry red ringspot virus
30 promoter, a ubiquitin gene promoter, an actin gene promoter, an NeIF-4A10
promoter, a maize Adh1-based pEmu promoter, a barley leaf thionin BTH6 promoter,
a cassava vein mosaic virus promoter, a sugarcane bacilliform badnavirus promoter
and a histone gene promoter.

180. The recombinant DNA molecule for construction of a vector for expressing a heterologous polypeptide in a transgenic cell of claim 179, wherein the eukaryotic constitutive promoter is a cauliflower mosaic virus 35S promoter.

181. The recombinant DNA molecule for construction of a vector for
5 expressing a heterologous polypeptide in a transgenic cell of claim 180, wherein the cauliflower mosaic virus 35S promoter comprises the sequence:
AGATTAGCCTTTTCAATTTTCAGAAAGAATGCTAACCCACAGATGGTTAGA
GAGGCTTACGCAGCAGGTCTCATCAAGACGATCTACCCGAGCAATAATCT
CCAGGAAATCAAATACCTTCCCAAGAAGGTTAAAGATGCAGTCAAAAGAT
10 TCAGGACTAACTGCATCAAGAACACAGAGAAAGATATATTTCTCAAGATC
AGAAGTACTATTCCAGTATGGACGATTCAAGGCTTGCTTCACAAACCAAG
GCAAGTAATAGAGATTGGAGTCTCTAAAAAGGTAGTTCCCACTGAATCAA
AGGCCATGGAGTCAAAGATTCAAATAGAGGACCTAACAGAACTCGCCGTA
AAGACTGGCGAACAGTTCATACAGAGTCTCTTACGACTCAATGACAAGAA
15 GAAAATCTTCGTCAACATGGTGGAGCACGACACACTTGTCTACTCCAAAA
ATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTGAGACTTTTCAA
CAAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTGCCAGCTATCTGT
CACTTTATTGTGAAGATAGTGGAAGGAAGGTGGCTCCTACAAATGCCA
TCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTG
20 GTCCCAAAGATGGACCCCCACCCACGAGGAGCATCGTGGAAGAAAGAAGA
CGTTCCAACCACGTCTTCAAAGCAAGTGGATTGATGTGATATCTCCACTGA
CGTAAGGGATGACGCACAATCCCACTATCCTTCGCAAGACCCTTCCTCTAT
ATAAGGAAGTTCATTTCAATTTGGAGAGAACACG (SEQ ID NO: 3).

182. The recombinant DNA molecule for construction of a vector for
25 expressing a heterologous polypeptide in a transgenic cell of claim 176, wherein the coding sequence for a heterologous polypeptide encodes a polypeptide selected from the group consisting of a hormone, an enzyme, a cell toxin, a viral polypeptide, a cell surface polypeptide, and an intracellular polypeptide.

183. The recombinant DNA molecule for construction of a vector for
30 expressing a heterologous polypeptide in a transgenic cell of claim 176, wherein the sequence complementary to an internal ribosome entry site is a sequence complementary to an IRES selected from the group consisting of a picornavirus IRES, a foot-and-mouth disease virus IRES, an encephalomyocarditis virus IRES, a hepatitis A virus IRES, a hepatitis C virus IRES, a human rhinovirus IRES, a poliovirus IRES,

a swine vesicular disease virus IRES, a turnip mosaic potyvirus IRES, a human fibroblast growth factor 2 mRNA IRES, a pestivirus IRES, a *Leishmania* RNA virus IRES, a Moloney murine leukemia virus IRES a human rhinovirus 14 IRES, an aphthovirus IRES, a human immunoglobulin heavy chain binding protein mRNA IRES, a *Drosophila* Antennapedia mRNA IRES, a human fibroblast growth factor 2 mRNA IRES, a hepatitis G virus IRES, a tobamovirus IRES, a vascular endothelial growth factor mRNA IRES, a Coxsackie B group virus IRES, a c-myc protooncogene mRNA IRES, a human MYT2 mRNA IRES, a human parechovirus type 1 virus IRES, a human parechovirus type 2 virus IRES, a eukaryotic initiation factor 4GI mRNA IRES, a *Plautia stali* intestine virus IRES, a Theiler's murine encephalomyelitis virus IRES, a bovine enterovirus IRES, a connexin 43 mRNA IRES, a homeodomain protein Gtx mRNA IRES, an AML1 transcription factor mRNA IRES, an NF-kappa B repressing factor mRNA IRES, an X-linked inhibitor of apoptosis mRNA IRES, a cricket paralysis virus RNA IRES, a p58(PITSLRE) protein kinase mRNA IRES, an ornithine decarboxylase mRNA IRES, a connexin-32 mRNA IRES, a bovine viral diarrhea virus IRES, an insulin-like growth factor I receptor mRNA IRES, a human immunodeficiency virus type 1 gag gene IRES, a classical swine fever virus IRES, a Kaposi's sarcoma-associated herpes virus IRES, a short IRES selected from a library of random oligonucleotides, a Jembrana disease virus IRES, an apoptotic protease-activating factor 1 mRNA IRES, a *Rhopalosiphum padi* virus IRES, a cationic amino acid transporter mRNA IRES, a human insulin-like growth factor II leader 2 mRNA IRES, a giardavirus IRES, a Smad5 mRNA IRES, a porcine teschovirus-1 talfan IRES, a *Drosophila* Hairless mRNA IRES, an hSNM1 mRNA IRES, a Cbfa1/Runx2 mRNA IRES, an Epstein-Barr virus IRES, a hibiscus chlorotic ringspot virus IRES, a rat pituitary vasopressin V1b receptor mRNA IRES, and a human hsp70 mRNA IRES.

184. The recombinant DNA molecule for construction of a vector for expressing a heterologous polypeptide in a transgenic cell of claim 183, wherein the sequence complementary to an internal ribosome entry site is a sequence complementary to a picornavirus internal ribosome entry site.

185. The recombinant DNA molecule for construction of a vector for expressing a heterologous polypeptide in a transgenic cell of claim 184, wherein the sequence complementary to a picornavirus internal ribosome entry site comprises the sequence:

TTATCATCGTGTGTTTTTCAAAGGAAAACACGTCCCCGTGGTTCGGGGGGGCC
TAGACGTTTTTTTAAACCTCGACTAAACACATGTAAAGCATGTGCACCGAG
GCCCCAGATCAGATCCCATAACAATGGGGTACCTTCTGGGCATCCTTCAGCC
CCTTGTGTAATACGCTTGAGGAGAGCCATTTGACTCTTTCCACAACATATCC
5 AACTCACAACGTGGCACTGGGGTTGTGCCGCCTTTGCAGGTGTATCTTATA
CACGTGGCTTTTGGCCGCAGAGGCACCTGTCGCCAGGTGGGGGGTTCCGC
TGCCTGCAAAGGGTCGCTACAGACGTTGTTTGTCTTCAAGAAGCTTCCAGA
GGAAGTCTTCCTTCACGACATTCAACAGACCTTGCATTCCTTTGGCGAGA
GGGGAAAGACCCCTAGGAATGCTCGTCAAGAAGACAGGGGCCAGGTTTCC
10 GGGCCCTCACATTGCCAAAAGACGGCAATATGGTGGAAAATCACATATAG
ACAAACGCACACCGGCCTTATTCCAAGCGGCTTCGGCCAGTAACGTTAGG
GGGGGGGGAGGGAGAGGGGCGGAATT (SEQ ID NO: 6).

186. The recombinant DNA molecule for construction of a vector for
expressing a heterologous polypeptide in a transgenic cell of claim 176, wherein the 3'
15 UTR of a positive strand single-stranded RNA virus is a 3' UTR of a positive strand
single-stranded RNA virus having no DNA stage.

187. The recombinant DNA molecule for construction of a vector for
expressing a heterologous polypeptide in a transgenic cell of claim 186, wherein the 3'
UTR of a positive strand single-stranded RNA virus having no DNA stage is a 3' UTR
20 of a bromovirus.

188. The recombinant DNA molecule for construction of a vector for
expressing a heterologous polypeptide in a transgenic cell of claim 187, wherein the 3'
UTR of a bromovirus is a 3' UTR of a Cowpea chlorotic mottle virus.

189. The recombinant DNA molecule for construction of a vector for
25 expressing a heterologous polypeptide in a transgenic cell of claim 188, wherein a
DNA copy of the 3' UTR of a Cowpea chlorotic mottle virus comprises the sequence:
AGTGCCCGCTGAAGAGCGTTACACTAGTGTGGCCTACTTGAAGGCTAGTT
ATAACCGTTTCTTTAAACGGTAATCGTTGTTGAAACGTCTTCCTTTTACAA
GAGGATTGAGCTGCCCTTGGGTTTACTCCTTGAACCCTTCGGAAGAACTC
30 TTTGGAGTTCGTACCAGTACCTCACATAGTGAGGTAATAAGACTGGTGGG
CAGCGCCTAGTCGAAAGACTAGGTGATCTCTAAGGAGACC (SEQ ID NO:
8).

190. The recombinant DNA molecule for construction of a vector for expressing a heterologous polypeptide in a transgenic cell of claim 176, further comprising a sequence complementary to an intron.

5 191. The recombinant DNA molecule for construction of a vector for expressing a heterologous polypeptide in a transgenic cell of claim 176, further comprising a transcription termination signal.

192. The recombinant DNA molecule for construction of a vector for expressing a heterologous polypeptide in a transgenic cell of claim 176, wherein the at least one site for insertion of a sequence comprising coding sequence of a
10 heterologous polypeptide in an antisense orientation comprises a recombination site.

193. The recombinant DNA molecule for construction of a vector for expressing a heterologous polypeptide in a transgenic cell of claim 192, wherein the recombination site is selected from the group consisting of a bacteriophage lambda *att* site and a topoisomerase I-based recombination site.

15 194. The recombinant DNA molecule for construction of a vector for expressing a heterologous polypeptide in a transgenic cell of claim 176, wherein the at least one site for insertion of a sequence comprising coding sequence of a heterologous polypeptide in an antisense orientation comprises at least one restriction enzyme recognition site.

20 195. The recombinant DNA molecule for construction of a vector for expressing a heterologous polypeptide in a transgenic cell of claim 176, wherein the at least one restriction enzyme recognition site comprises a polylinker.

196. A method for making a transgenic vector for expression of a heterologous polypeptide in a transgenic cell, the method comprising:

25 a) providing a DNA molecule for construction of a vector for expressing a heterologous polypeptide in a transgenic cell, the DNA molecule comprising a promoter operably linked, in the 5' to 3' direction, to a DNA sequence comprising:

30 i) at least one site for insertion of a sequence comprising coding sequence of a heterologous polypeptide in an antisense orientation;

ii) a sequence complementary to an internal ribosome entry site; and

iii) a 3' UTR of a positive strand single-stranded RNA virus; and

b) inserting a sequence encoding a heterologous polypeptide into the insertion site of the DNA molecule in an antisense orientation relative to the direction of transcription from the promoter.

5

197. The method for making a transgenic vector for expression of a heterologous polypeptide in a transgenic cell of claim 196, wherein the promoter is a selected from the group consisting of a constitutive promoter and an inducible promoter.

10

198. The method for making a transgenic vector for expression of a heterologous polypeptide in a transgenic cell of claim 197, wherein the promoter is a constitutive promoter.

15

199. The method for making a transgenic vector for expression of a heterologous polypeptide in a transgenic cell of claim 198, wherein the constitutive promoter is a eukaryotic constitutive promoter selected from the group consisting of a cauliflower mosaic virus 35S promoter, a blueberry red ringspot virus promoter, a ubiquitin gene promoter, an actin gene promoter, an NeIF-4A10 promoter, a maize Adh1-based pEmu promoter, a barley leaf thionin BTH6 promoter, a cassava vein mosaic virus promoter, a sugarcane bacilliform badnavirus promoter and a histone gene promoter.

20

200. The method for making a transgenic vector for expression of a heterologous polypeptide in a transgenic cell of claim 199, wherein the eukaryotic constitutive promoter is a cauliflower mosaic virus 35S promoter.

25

201. The method for making a transgenic vector for expression of a heterologous polypeptide in a transgenic cell of claim 200, wherein the cauliflower mosaic virus 35S promoter comprises the sequence:

AGATTAGCCTTTTCAATTTTCAGAAAGAATGCTAACCCACAGATGGTTAGA
GAGGCTTACGCAGCAGGTCTCATCAAGACGATCTACCCGAGCAATAATCT
CCAGGAAATCAAATACCTTCCCAAGAAGGTTAAAGATGCAGTCAAAAGAT
30 TCAGGACTAACTGCATCAAGAACACAGAGAAAGATATATTTCTCAAGATC
AGAAGTACTATTCCAGTATGGACGATTCAAGGCTTGCTTCACAAACCAAG
GCAAGTAATAGAGATTGGAGTCTCTAAAAAGGTAGTTCCCACTGAATCAA
AGGCCATGGAGTCAAAGATTCAAATAGAGGACCTAACAGAACTCGCCGTA
AAGACTGGCGAACAGTTCATACAGAGTCTCTTACGACTCAATGACAAGAA

GAAAATCTTCGTCAACATGGTGGAGCACGACACACTTGTCTACTCCAAAA
 ATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTGAGACTTTTCAA
 CAAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGT
 CACTTTATTGTGAAGATAGTGGAAAAGGAAGGTGGCTCCTACAAATGCCA
 5 TCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTG
 GTCCCAAAGATGGACCCCCACCCACGAGGAGCATCGTGGAAAAAGAAGA
 CGTTCCAACCACGTCTTCAAAGCAAGTGGATTGATGTGATATCTCCACTGA
 CGTAAGGGATGACGCACAATCCCACTATCCTTCGCAAGACCCTTCCTCTAT
 ATAAGGAAGTTCATTTCAATTTGGAGAGAACACG (SEQ ID NO: 3).

10 202. The method for making a transgenic vector for expression of a heterologous polypeptide in a transgenic cell of claim 196, wherein the coding sequence for a heterologous polypeptide encodes a polypeptide selected from the group consisting of a hormone, an enzyme, a cell toxin, a viral polypeptide, a cell surface polypeptide, and an intracellular polypeptide.

15 203. The method for making a transgenic vector for expression of a heterologous polypeptide in a transgenic cell of claim 196, wherein the sequence complementary to an internal ribosome entry site is a sequence complementary to an IRES selected from the group consisting of a picornavirus IRES, a foot-and-mouth disease virus IRES, an encephalomyocarditis virus IRES, a hepatitis A virus IRES, a
 20 hepatitis C virus IRES, a human rhinovirus IRES, a poliovirus IRES, a swine vesicular disease virus IRES, a turnip mosaic potyvirus IRES, a human fibroblast growth factor 2 mRNA IRES, a pestivirus IRES, a Leishmania RNA virus IRES, a Moloney murine leukemia virus IRES a human rhinovirus 14 IRES, an aphthovirus IRES, a human immunoglobulin heavy chain binding protein mRNA IRES, a
 25 *Drosophila* Antennapedia mRNA IRES, a human fibroblast growth factor 2 mRNA IRES, a hepatitis G virus IRES, a tobamovirus IRES, a vascular endothelial growth factor mRNA IRES, a Cocksackie B group virus IRES, a c-myc protooncogene mRNA IRES, a human MYT2 mRNA IRES, a human parechovirus type 1 virus IRES, a human parechovirus type 2 virus IRES, a eukaryotic initiation factor 4GI mRNA
 30 IRES, a *Plautia stali* intestine virus IRES, a Theiler's murine encephalomyelitis virus IRES, a bovine enterovirus IRES, a connexin 43 mRNA IRES, a homeodomain protein Gtx mRNA IRES, an AML1 transcription factor mRNA IRES, an NF-kappa B repressing factor mRNA IRES, an X-linked inhibitor of apoptosis mRNA IRES, a cricket paralysis virus RNA IRES, a p58(PITSLRE) protein kinase mRNA IRES, an

ornithine decarboxylase mRNA IRES, a connexin-32 mRNA IRES, a bovine viral diarrhea virus IRES, an insulin-like growth factor I receptor mRNA IRES, a human immunodeficiency virus type 1 gag gene IRES, a classical swine fever virus IRES, a Kaposi's sarcoma-associated herpes virus IRES, a short IRES selected from a library of random oligonucleotides, a Jembrana disease virus IRES, an apoptotic protease-activating factor 1 mRNA IRES, a Rhopalosiphum padi virus IRES, a cationic amino acid transporter mRNA IRES, a human insulin-like growth factor II leader 2 mRNA IRES, a giardiavirus IRES, a Smad5 mRNA IRES, a porcine teschovirus-1 talfan IRES, a *Drosophila* Hairless mRNA IRES, an hSNM1 mRNA IRES, a Cbfa1/Runx2 mRNA IRES, an Epstein-Barr virus IRES, a hibiscus chlorotic ringspot virus IRES, a rat pituitary vasopressin V1b receptor mRNA IRES, and a human hsp70 mRNA IRES.

204. The method for making a transgenic vector for expression of a heterologous polypeptide in a transgenic cell of claim 203, wherein the sequence complementary to an internal ribosome entry site is a sequence complementary to a picornavirus internal ribosome entry site.

205. The method for making a transgenic vector for expression of a heterologous polypeptide in a transgenic cell of claim 204, wherein the sequence complementary to a picornavirus internal ribosome entry site comprises the sequence:

TTATCATCGTGTTTTTCAAAGGAAAACACGTCCTCCCGTGTTTCGGGGGGGCC
TAGACGTTTTTTTAACTCGACTAAACACATGTAAAGCATGTGCACCGAG
GCCCCAGATCAGATCCCATAACAATGGGGTACCTTCTGGGCATCCTTCAGCC
CCTTGTTGAATACGCTTGAGGAGAGCCATTTGACTCTTTCCACAACATATCC
AACTCACAACGTGGCACTGGGGTTGTGCCGCCTTTGCAGGTGTATCTTATA
CACGTGGCTTTTGGCCGCAGAGGCACCTGTCGCCAGGTGGGGGGTTCCGC
TGCCTGCAAAGGGTCGCTACAGACGTTGTTTGTCTTCAAGAAGCTTCCAGA
GGAAGTGTCTTCTTCACGACATTCAACAGACCTTGCATTCTTTGGCGAGA
GGGGAAAGACCCCTAGGAATGCTCGTCAAGAAGACAGGGCCAGGTTTCC
GGGCCCTCACATTGCCAAAAGACGGCAATATGGTGGAAAATCACATATAG
ACAAACGCACACCGGCCTTATTCCAAGCGGCTTCGGCCAGTAACGTTAGG
GGGGGGGGAGGGAGAGGGGCGGAATT (SEQ ID NO: 6).

206. The method for making a transgenic vector for expression of a heterologous polypeptide in a transgenic cell of claim 196, wherein the 3' UTR of a

positive strand single-stranded RNA virus is a 3' UTR of a positive strand single-stranded RNA virus having no DNA stage.

207. The method for making a transgenic vector for expression of a heterologous polypeptide in a transgenic cell of claim 206, wherein the 3' UTR of a positive strand single-stranded RNA virus having no DNA stage is a 3' UTR of a bromovirus.

208. The method for making a transgenic vector for expression of a heterologous polypeptide in a transgenic cell of claim 207, wherein the 3' UTR of a bromovirus is a 3' UTR of a Cowpea chlorotic mottle virus.

209. The method for making a transgenic vector for expression of a heterologous polypeptide in a transgenic cell of claim 208, wherein a DNA copy of the 3' UTR of a Cowpea chlorotic mottle virus comprises the sequence:
AGTGCCCGCTGAAGAGCGTTACACTAGTGTGGCCTACTTGAAGGCTAGTT
ATAACCGTTTCTTTAAACGGTAATCGTTGTTGAAACGTCTTCCTTTTACAA
GAGGATTGAGCTGCCCTTGGGTTTTACTCCTTGAACCCTTCGGAAGAAGCTC
TTTGGAGTTCGTACCAGTACCTCACATAGTGAGGTAATAAGACTGGTGGG
CAGCGCCTAGTCGAAAGACTAGGTGATCTCTAAGGAGACC (SEQ ID NO: 8).

210. The method for making a transgenic vector for expression of a heterologous polypeptide in a transgenic cell of claim 196, further comprising a sequence complementary to an intron.

211. The method for making a transgenic vector for expression of a heterologous polypeptide in a transgenic cell of claim 196, further comprising a transcription termination signal.

212. The method for making a transgenic vector for expression of a heterologous polypeptide in a transgenic cell of claim 196, wherein the at least one site for insertion of a sequence comprising coding sequence of a heterologous polypeptide in an antisense orientation comprises a recombination site.

213. The method for making a transgenic vector for expression of a heterologous polypeptide in a transgenic cell of claim 212, wherein the recombination site is selected from the group consisting of a bacteriophage lambda *att* site and a topoisomerase I-based recombination site.

214. The method for making a transgenic vector for expression of a heterologous polypeptide in a transgenic cell of claim 196, wherein the at least one

site for insertion of a sequence comprising coding sequence of a heterologous polypeptide in an antisense orientation comprises at least one restriction enzyme recognition site.

215. The method for making a transgenic vector for expression of a heterologous polypeptide in a transgenic cell of claim 196, wherein the at least one restriction enzyme recognition site comprises a polylinker.

216. A kit for constructing a vector for expressing a heterologous polypeptide in a transgenic cell, the kit comprising a DNA molecule for construction of a vector for expressing a heterologous polypeptide in a transgenic cell, the DNA molecule comprising a promoter operably linked, in the 5' to 3' direction, to a DNA sequence comprising:

- a) at least one site for insertion of a sequence comprising coding sequence of a heterologous polypeptide in an antisense orientation;
- b) a sequence complementary to an internal ribosome entry site;
- and
- c) a 3' UTR of a positive strand single-stranded RNA virus.

217. The kit for constructing a vector for expressing a heterologous polypeptide in a transgenic cell of claim 216, wherein the promoter is a selected from the group consisting of a constitutive promoter and an inducible promoter.

218. The kit for constructing a vector for expressing a heterologous polypeptide in a transgenic cell of claim 217, wherein the promoter is a constitutive promoter.

219. The kit for constructing a vector for expressing a heterologous polypeptide in a transgenic cell of claim 218, wherein the constitutive promoter is a eukaryotic constitutive promoter selected from the group consisting of a cauliflower mosaic virus 35S promoter, a blueberry red ringspot virus promoter, a ubiquitin gene promoter, an actin gene promoter, an NeIF-4A10 promoter, a maize Adh1-based pEmu promoter, a barley leaf thionin BTH6 promoter, a cassava vein mosaic virus promoter, a sugarcane bacilliform badnavirus promoter and a histone gene promoter.

220. The kit for constructing a vector for expressing a heterologous polypeptide in a transgenic cell of claim 219, wherein the eukaryotic constitutive promoter is a cauliflower mosaic virus 35S promoter.

221. The kit for constructing a vector for expressing a heterologous polypeptide in a transgenic cell of claim 220, wherein the cauliflower mosaic virus

35S promoter comprises the sequence:

AGATTAGCCTTTTCAATTTTCAGAAAGAATGCTAACCCACAGATGGTTAGA
 GAGGCTTACGCAGCAGGTCTCATCAAGACGATCTACCCGAGCAATAATCT
 CCAGGAAATCAAATACCTTCCCAAGAAGGTTAAAGATGCAGTCAAAAGAT
 5 TCAGGACTAACTGCATCAAGAACACAGAGAAAGATATATTTTCTCAAGATC
 AGAAGTACTATTCCAGTATGGACGATTCAAGGCTTGCTTCACAAACCAAG
 GCAAGTAATAGAGATTGGAGTCTCTAAAAAGGTAGTTCCCACTGAATCAA
 AGGCCATGGAGTCAAAGATTCAAATAGAGGACCTAACAGAACTCGCCGTA
 AAGACTGGCGAACAGTTCATACAGAGTCTCTTACGACTCAATGACAAGAA
 10 GAAAATCTTCGTCAACATGGTGGAGCACGACACACTTGTCTACTCCAAAA
 ATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTGAGACTTTTCAA
 CAAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTGCCAGCTATCTGT
 CACTTTATTGTGAAGATAGTGGAAAAGGAAGGTGGCTCCTACAAATGCCA
 TCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTG
 15 GTCCCAAAGATGGACCCCCACCCACGAGGAGCATCGTGGA AAAAAGAAGA
 CGTTCCAACCACGTCTTCAAAGCAAGTGGATTGATGTGATATCTCCACTGA
 CGTAAGGGATGACGCACAATCCCACTATCCTTCGCAAGACCCTTCCTCTAT
 ATAAGGAAGTTCATTTCAATTTGGAGAGAACACG (SEQ ID NO: 3).

222. The kit for constructing a vector for expressing a heterologous
 20 polypeptide in a transgenic cell of claim 216, wherein the coding sequence for a
 heterologous polypeptide encodes a polypeptide selected from the group consisting of
 a hormone, an enzyme, a cell toxin, a viral polypeptide, a cell surface polypeptide,
 and an intracellular polypeptide.

223. The kit for constructing a vector for expressing a heterologous
 25 polypeptide in a transgenic cell of claim 216, wherein the sequence complementary to
 an internal ribosome entry site is a sequence complementary to an IRES selected from
 the group consisting of a picornavirus IRES, a foot-and-mouth disease virus IRES, an
 encephalomyocarditis virus IRES, a hepatitis A virus IRES, a hepatitis C virus IRES,
 a human rhinovirus IRES, a poliovirus IRES, a swine vesicular disease virus IRES, a
 30 turnip mosaic potyvirus IRES, a human fibroblast growth factor 2 mRNA IRES, a
 pestivirus IRES, a Leishmania RNA virus IRES, a Moloney murine leukemia virus
 IRES a human rhinovirus 14 IRES, an aphthovirus IRES, a human immunoglobulin
 heavy chain binding protein mRNA IRES, a *Drosophila* Antennapedia mRNA IRES,
 a human fibroblast growth factor 2 mRNA IRES, a hepatitis G virus IRES, a

tobamovirus IRES, a vascular endothelial growth factor mRNA IRES, a Coxsackie B group virus IRES, a c-myc protooncogene mRNA IRES, a human MYT2 mRNA IRES, a human parechovirus type 1 virus IRES, a human parechovirus type 2 virus IRES, a eukaryotic initiation factor 4GI mRNA IRES, a *Plautia stali* intestine virus IRES, a Theiler's murine encephalomyelitis virus IRES, a bovine enterovirus IRES, a connexin 43 mRNA IRES, a homeodomain protein Gtx mRNA IRES, an AML1 transcription factor mRNA IRES, an NF-kappa B repressing factor mRNA IRES, an X-linked inhibitor of apoptosis mRNA IRES, a cricket paralysis virus RNA IRES, a p58(PITSLRE) protein kinase mRNA IRES, an ornithine decarboxylase mRNA IRES, a connexin-32 mRNA IRES, a bovine viral diarrhea virus IRES, an insulin-like growth factor I receptor mRNA IRES, a human immunodeficiency virus type 1 gag gene IRES, a classical swine fever virus IRES, a Kaposi's sarcoma-associated herpes virus IRES, a short IRES selected from a library of random oligonucleotides, a Jembrana disease virus IRES, an apoptotic protease-activating factor 1 mRNA IRES, a *Rhopalosiphum padi* virus IRES, a cationic amino acid transporter mRNA IRES, a human insulin-like growth factor II leader 2 mRNA IRES, a giardiavirus IRES, a Smad5 mRNA IRES, a porcine teschovirus-1 talfan IRES, a *Drosophila* Hairless mRNA IRES, an hSNM1 mRNA IRES, a Cbfa1/Runx2 mRNA IRES, an Epstein-Barr virus IRES, a hibiscus chlorotic ringspot virus IRES, a rat pituitary vasopressin V1b receptor mRNA IRES, and a human hsp70 mRNA IRES.

224. The kit for constructing a vector for expressing a heterologous polypeptide in a transgenic cell of claim 223, wherein the sequence complementary to an internal ribosome entry site is a sequence complementary to a picornavirus internal ribosome entry site.

225. The kit for constructing a vector for expressing a heterologous polypeptide in a transgenic cell of claim 224, wherein the sequence complementary to a picornavirus internal ribosome entry site comprises the sequence:

TTATCATCGTGTTTTTCAAAGGAAAACACGTCCCCGTGGTTCGGGGGGGCC
TAGACGTTTTTTTAACCTCGACTAAACACATGTAAAGCATGTGCACCGAG
GCCCCAGATCAGATCCCATAACAATGGGGTACCTTCTGGGCATCCTTCAGCC
CCTTGTTGAATACGCTTGAGGAGAGCCATTTGACTCTTTCCACAACATCC
AACTCACAACGTGGCACTGGGGTTGTGCCGCCTTTGCAGGTGTATCTTATA
CACGTGGCTTTTGGCCGCAGAGGCACCTGTCGCCAGGTGGGGGGTTCCGC
TGCCTGCAAAGGGTCGCTACAGACGTTGTTTGTCTTCAAGAAGCTTCCAGA

GGAAGTGGCTTCCTTCACGACATTCAACAGACCTTGCATTCCTTTGGCGAGA
GGGGAAAGACCCCTAGGAATGCTCGTCAAGAAGACAGGGCCAGGTTTCC
GGGCCCTCACATTGCCAAAAGACGGCAATATGGTGGAAAATCACATATAG
ACAAACGCACACCGGCCTTATTCCAAGCGGCTTCGGCCAGTAACGTTAGG
5 GGGGGGGGAGGGAGAGGGGCGGAATT (SEQ ID NO: 6).

226. The kit for constructing a vector for expressing a heterologous polypeptide in a transgenic cell of claim 216, wherein the 3' UTR of a positive strand single-stranded RNA virus is a 3' UTR of a positive strand single-stranded RNA virus having no DNA stage.

10 227. The kit for constructing a vector for expressing a heterologous polypeptide in a transgenic cell of claim 226, wherein the 3' UTR of a positive strand single-stranded RNA virus having no DNA stage is a 3' UTR of a bromovirus.

228. The kit for constructing a vector for expressing a heterologous polypeptide in a transgenic cell of claim 227, wherein the 3' UTR of a bromovirus is a
15 3' UTR of a Cowpea chlorotic mottle virus.

229. The kit for constructing a vector for expressing a heterologous polypeptide in a transgenic cell of claim 228, wherein a DNA copy of the 3' UTR of a Cowpea chlorotic mottle virus comprises the sequence:
AGTGCCCGCTGAAGAGCGTTACACTAGTGTGGCCTACTTGAAGGCTAGTT
20 ATAACCGTTTCTTTAAACGGTAATCGTTGTTGAAACGTCTTCCTTTTACAA
GAGGATTGAGCTGCCCTTGGGTTTACTCCTTGAACCCTTCGGAAGAAGCTC
TTTGGAGTTCGTACCAAGTACCTCACATAGTGAGGTAATAAGACTGGTGGG
CAGCGCCTAGTCGAAAGACTAGGTGATCTCTAAGGAGACC (SEQ ID NO:
8).

25 230. The kit for constructing a vector for expressing a heterologous polypeptide in a transgenic cell of claim 216, further comprising a sequence complementary to an intron.

231. The kit for constructing a vector for expressing a heterologous polypeptide in a transgenic cell of claim 216, further comprising a transcription
30 termination signal.

232. The kit for constructing a vector for expressing a heterologous polypeptide in a transgenic cell of claim 216, wherein the at least one site for insertion of a sequence comprising coding sequence of a heterologous polypeptide in an antisense orientation comprises a recombination site.

233. The kit for constructing a vector for expressing a heterologous polypeptide in a transgenic cell of claim 232, wherein the recombination site is selected from the group consisting of a bacteriophage lambda *att* site and a topoisomerase I-based recombination site.

5 234. The kit for constructing a vector for expressing a heterologous polypeptide in a transgenic cell of claim 216, wherein the at least one site for insertion of a sequence comprising coding sequence of a heterologous polypeptide in an antisense orientation comprises at least one restriction enzyme recognition site.

10 235. The kit for constructing a vector for expressing a heterologous polypeptide in a transgenic cell of claim 216, wherein the at least one restriction enzyme recognition site comprises a polylinker.